University of Alberta

Aspects of Nutrition and Metabolism in the Periparturient Sow

by

Helen Margaret Miller ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Permanent Address: 534, Michener Park,
Edmonton, Alberta,
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Dated 15th August 1996

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ASPECTS OF NUTRITION AND METABOLISM IN THE PERIPARTURIENT SOW submitted by HELEN MARGARET MILLER in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL NUTRITION.

Frank Aherne
F.X. Aherne

G.R. Foxcroft

Walt Dixon
W.T. Dixon

Al Schaefer
A.L. Schaefer

V Harber
V. Harber

J.E. Pettigrew

Date: Aug 14, 1996

Dedication

To Lawrie, Michael and Robbie without whose support, encouragement and understanding this thesis could not have been completed.

Abstract

The overall aims of this thesis were to investigate how sows responded to different levels of feed intake during the periparturient period and to investigate the nutritional regulation of progesterone and prolactin during lactogenesis and lactation. In experiment 1, 40 progesterone-infused ovariectomised Camborough x Canabrid gilts were used to determine the effects of different feed intakes (2.3 versus 1.15 times maintenance (M)) and different fatness (10 versus 20 mm backfat) on metabolic clearance rate (MCR) and half-life of progesterone. Increasing feed intake increased MCR of progesterone and tended to reduce half-life. Gilt fatness did not affect progesterone kinetics.

In experiment 2, 117 sows (parities 1-3) were fed high (2M) versus normal (1.15M) intakes in late gestation to establish whether plasma progesterone could be reduced by increased feed intake approaching farrowing and hence induce early onset of lactogenesis. Feed intake in late gestation did not affect progesterone concentration or time of onset of lactogenesis. In contrast to experiment 1, half-life of progesterone was longer in fatter sows. High intake sows lost less backfat between d98 of gestation and weaning but otherwise sow performance was similar for both treatments.

Experiment 3 investigated feeding *ad libitum* (AL) versus restricted (1M week 1, 1.5M week 2) to 16 primiparous sows during a 2 week lactation. Feed intake did not affect progesterone or prolactin concentrations nor timing of lactogenesis. Insulin and IGF-1 concentrations were higher in AL sows from day 3 of lactation. Restricted sows lost 16.3kg and 2.5mm backfat in week1 versus 2.5kg and 1.3mm for AL sows, and piglet growth rates were similar. In week2 liveweight and backfat losses were similar between treatments but piglets from AL sows grew faster. Abundance of messenger ribonucleic acid (mRNA) for progesterone receptor in mammary gland decreased between 5d prefarrowing and 2d postfarrowing and was absent in established lactation. Mammary gland prolactin receptor mRNA abundance increased from prefarrowing through to established lactation. Prolactin receptor mRNA abundance was not affected by feed intake but did exert an independent positive effect on milk production. Mammary gland GLUT1 mRNA abundance increased from prefarrowing through to established lactation and increased with both feed intake and milk production. GLUT1 has an important role in determining milk yield.

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List of Abbreviations

Abbreviation	**Meaning**
AL	*Ad libitum*
BW	body weight
BSA	bovine serum albumin
CBG	cortisol binding globulin
DE	digestible energy
F	fat
FSH	follicle stimulating hormone
GB	Golgi bodies
GH	growth hormone
GLUT	glucose transporter
GnRH	gonadotrophin-releasing hormone
GRF	growth hormone-releasing factor
H	high feed intake
IGF-1	insulin-like growth factor-1
k	disappearance rate constant
L	low feed intake
LH	luteinising hormone
LHRH	luteinising hormone releasing hormone
Ln	lean
MCR	metabolic clearance rate

ME	metabolisable energy
MFO	mixed function oxidases
N	normal feed intake
R	restricted
RER	rough endoplasmic reticulum
TRF	thyrotropin-releasing factor
WEI	weaning to estrus interval

CHAPTER 1

1.1 Introduction

This study focuses on the periparturient sow and investigates how her productivity might be manipulated by feed intake. The periparturient period involves dramatic metabolic change as the sow switches from nutrient transfer via the placenta to the fetus to highly efficient milk production. Over a short period the sow must produce a viable litter of piglets, establish and maintain lactation and prepare for her next pregnancy. For the purpose of this study the periparturient period was considered to be from d 100 of gestation until the end of the second week of lactation. This period will assume greater significance as segregated early weaning increases in popularity as a production strategy in which there is less time for the sow to compensate for any inadequacies in periparturient performance.

1.2 Literature Review

Traditionally sows become catabolic in late gestation (Cole, 1990). The demands for fetal growth and mammary development during gestation are relatively small and, although most sows are also targeted to gain a certain amount of weight during pregnancy, the sow's major nutrient cost during gestation is her own maintenance requirement which represents 75 to 85% of her energy intake (Aherne and Williams, 1992). Noblet *et al.* (1985) calculated that the energy requirements for reproduction increased from 3 to 12% of maternal energy intake between d 50 and 110 of gestation whilst protein requirements for reproduction increased from 7 to 41% over the same period. Growth of the fetus increases exponentially throughout gestation, increasing from 66 g/d on d 60 of gestation to 240 g/d on d 110 (Noblet *et al.*, 1985). In addition when sows receive a constant daily amount of feed throughout pregnancy, as is frequently the case, their energy intake per unit body weight during gestation decreases as a result of their increase in body size (Close *et al.*, 1985). Therefore sows receiving a gestation allowance of less than 25 MJ digestible energy (DE)/d may lose body reserves, primarily fat, in the last 2 to 3 weeks of pregnancy (Close *et al.*, 1985; Cole, 1990; Noblet *et al.*, 1990) unless their feed intake is increased during this period. Close *et al.* (1985) found that sows receiving 20 MJ metabolisable energy (ME)/d were in negative energy balance by d 87 of gestation and were estimated to be losing 140g fat per d by term. Whittemore (1993) stressed the importance of maintaining at least 12 mm P2 backfat on the sow at weaning if sow reproductive performance is not to be compromised. Most sows lose backfat during lactation (Mullan, 1991; Aherne *et al.*, 1991) and with modern lean genotypes it may be critical to maintain sow fatness in late gestation in order to maximise sow output and longevity in the breeding herd. Therefore increasing feed intake during the last two weeks of gestation would appear to be a sensible management strategy. However, there is controversy regarding acceptance of this practice since some researchers have observed a significant increase in postpartum agalactia when sows were fed high feed intakes prior to farrowing (Göransson, 1989; Persson *et al.*, 1989) In addition it is well known that increasing gestation feed intake reduces lactation feed intake (Salmon-Legagneur and Rerat, 1962, Mullan and Williams, 1989; Weldon *et al.*, 1994). Many workers have shown that the majority of sows,especially those of parities one and two, fail to eat enough to meet their nutrient requirements during lactation and hence use up body reserves (reviewed by Williams and Mullan, 1989; Aherne *et al.*, 1991). These sows become catabolic and then, in primiparous sows in particular, exhibit suppressed reproductive performance post weaning (King and Williams, 1984; Hughes, 1989; Williams and Mullan, 1989). However, increasing sow feed intake in late gestation need not have a detrimental effect on lactation feed intake, it may in fact achieve the opposite effect. Matte *et al.* (1994) and Farmer *et al.* (1996) have shown that feeding a greater volume to sows during gestation, using high fibre ingredients, resulted in increased lactation feed intake compared to controls. Presumably this results from enlarged capacity of the digestive tract because both groups of sows received similar nutrient intakes. Therefore, increasing feed intake in late gestation might also increase the capacity of the digestive system and lead to greater lactation feed intakes and, or, improved diet digestibility (Zivkovic and Bowland, 1963). In Chapter 3 the effects of high feed intake versus normal feeding during the last 2 weeks of gestation were compared in order to investigate their effects on diet digestibility, feed intake and sow performance in lactation.

The outcome of a successful farrowing is a large litter of healthy viable piglets. Piglets are born with few body reserves. They contain less than 1.5% fat (7.6% dry matter) at birth (Widdowson, 1950; Manners and McCrea, 1963; Elsley, 1965), much of which is structural and therefore not available as an energy source (Mersmann, 1974). Piglets at birth also have only moderate glycogen reserves (Pettigrew, 1981). Since fat is not only an energy reserve but also an insulator in the pig, the new born piglet is highly susceptible to any environmental or lactational deficiencies. It is widely recognised that heavier piglets have a greater chance of survival than lighter piglets (Dyck and Swierstra, 1987). Clearly it would be advantageous to develop a management strategy to increase piglet birthweight and, or, body reserves at birth.

There is evidence to suggest that piglet birthweight increases linearly as gestation energy intake increases up to 30 to 40 MJ ME /d (Elsley *et al.*, 1969; Noblet *et al.*, 1985). Fetal piglets are growing most rapidly during late gestation and therefore this would appear to be the optimal time in which to increase sow feed intake in order to maximise fetal growth. Surprisingly, such attempts have been largely unsuccessful (Hillyer and Phillips, 1980; Stirling and Cline, 1986). However, Cromwell *et al.* (1989) did observe an increase of 50 g in piglet birthweight from sows which had received increased feed intake in late gestation for at least two reproductive cycles.

1.2.1 Alternative means of increasing piglet birthweight

An alternative approach to increase piglet birthweight has been the use of high fat sow diets in late gestation (Pettigrew, 1981; Stahly *et al.*,1981; Cieslak *et al.*,1983; Campion *et al.*, 1984; Bishop *et al.*,1985ab; Coffey *et al.*, 1987; Seerley, 1989). However, responses to such diets have been variable and unspectacular. Pettigrew

(1981) summarised the data up to 1980 and found that feeding supplemental fat to sows during late gestation did not affect piglet glycogen stores but did increase carcass fat marginally. Fat concentration in colostrum and milk was increased and this increased piglet survival in high mortality herds (more than 20%), particularly for small piglets (birthweight of less than 1.1 kg), providing that the sow ate at least 1 kg fat before farrowing. More recent work supports these conclusions (Stahly *et al.*,1981; Cieslak *et al.*,1983; Campion *et al.*, 1984; Bishop *et al.*,1985ab; Coffey *et al.*, 1987; Newcomb *et al.*,1991). Although Seerley (1989) found that supplementing a corn-soybean meal diet with 5 or 10% solid fat pellets from d 80 or d 100 of gestation, respectively, produced piglets with 41% more liver glycogen and 16% higher serum glucose at birth. These extra body reserves did not affect survival in his experiment but this was already at high levels averaging 90%. From these results it is clear that feeding supplemental fat during late gestation only affects piglet survival in high mortality herds and herds with a predominance of low birthweight piglets.

Since the simple strategy of changing sow feed or energy intake in late gestation proved ineffective in increasing piglet birthweights and body reserves, a number of workers have tried an alternative strategy to improve piglet survival by modifying sow metabolism (Bishop *et al*, 1985ab; Ezekwe and Martin, 1978; Ezekwe, 1986). Feeding a synthetic glucocorticoid to the sow from 9 d prepartum increased carcass fat content of piglets at birth but did not affect birthweight or piglet survival (Bishop *et al*, 1985a). However, piglets from supplemented sows exhibited enhanced fat deposition during the first 2 d of life (Bishop *et al.*, 1985b). Feeding copper, dichlorvos, medium chain triglycerides or 1,3-butanediol to sows during late gestation all improved piglet survival to weaning (Stahly *et al.*, 1985; Thacker, 1991; Azain, 1993). However, none of the four treatments affected birthweight and, although survival was increased, the treated herds normally had high mortality (around 20%). Azain (1993) noted that increased survival rate was most pronounced in piglets of less than 900 g birthweight. Ezekwe and Martin (1978) induced diabetes in sows by intravenous injection of alloxan on d 70 of gestation. The resulting piglets had significantly heavier livers containing significantly more glycogen than piglets from control sows (Ezekwe and Martin, 1978; Ezekwe, 1986). Piglets were killed on d 112 of gestation so survival to weaning could not be measured.

It seems therefore that even drastic measures such as making sows diabetic have little or no effect on piglet birthweight although survival of low birthweight piglets may be improved by the introduction of fat to the late gestation diet or by one of the other methods described.

1.2.2 Hormonal changes during the periparturient period

The periparturient period is characterised by dramatic changes in the hormonal milieu of the sow which stimulate both parturition and lactogenesis. Progesterone, which is the dominant hormone of pregnancy (Robertson and King, 1974; DeHoff *et al.*, 1986), starts to decline over the last few days before parturition and then drops sharply during the last 24 to 48 h and during farrowing (Molokwu and Wagner, 1973;

Robertson and King, 1974; Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975; Duggan *et al.*, 1982). Mean reported values for progesterone concentration during farrowing vary from 1.5 to 4.7 ng/ml (Robertson and King, 1974; Baldwin and Stabenfeldt, 1975; Duggan *et al.*, 1982). Within 24 h of farrowing progesterone is almost undetectable and it remains at this low level throughout lactation (Molokwu and Wagner, 1973; Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975; Duggan *et al.*, 1982).

As progesterone concentration declines the concentrations of a number of other hormones increase rapidly. Plasma concentrations of estradiol and total estrogens start to increase from around d 70 to 80 of gestation (Robertson and King, 1974; DeHoff *et al.*, 1986). This increase continues, culminating in a peak during the last few days of gestation. Since estrogens are secreted by the placenta of the sow during pregnancy their concentrations decline rapidly after parturition (Molokwu and Wagner, 1973; Robertson and King, 1974; Ash and Heap, 1975; Duggan *et al.*, 1982).

Plasma prolactin concentrations remain at a steady level throughout gestation (4.7 to 10.7 ng/ml) but increase dramatically from 2 d prepartum and peak during farrowing reaching concentrationsas high as 147 ng/ml (Dusza and Krzymowska, 1981). Prolactin concentration declines gradually over the first 21 d of lactation (Plaut *et al.*, 1989) but remains at higher concentrations than the basal concentrations observed during the estrous cycle (Dusza and Tilton, 1990). Corticosteroids (Molokwu and Wagner, 1973; Ash and Heap, 1975), relaxin (Sherwood *et al.*, 1975), prostaglandin $F_2\alpha$ metabolites (Nara and First, 1981) and oxytocin (Forsling *et al.*, 1977) also peak during the periparturient period.

These dramatic changes in the hormone milieu bring about parturition and lactogenesis and since both occur concurrently in the sow it has been difficult to isolate the essential requirements for successful lactogenesis in this species. It is not the purpose of this thesis to discuss the hormonal control of parturition as this has been adequately reviewed elsewhere (First and Bosc, 1979; First *et al.*, 1982; Guthrie, 1985).

1.2.3 Mammogenesis and lactogenesis

1.2.3.1 Mammogenesis

Before lactogenesis can take place mammogenesis must occur. Basic mammary structure is already present at birth and some ductal development and stromal invasion does continue prepuberty (Forsyth, 1989). However, in gilts mammary development remains rudimentary until the second half of pregnancy (Hacker and Hill, 1972). Kensinger *et al.* (1982) noted some duct growth between d 30 and 60 of gestation followed by a dramatic increase in mammary epithelial cell concentration between d 60 and 75. By day 90 the mammary gland appeared to be fully differentiated for milk synthesis although there was little accumulation of colostrum in alveolar lumina. This period of mammary growth coincides with increasing plasma estrogen concentration in the pig (Robertson and King, 1974). In late pregnancy estrogens are primarily produced by the feto-placental unit (Craig, 1982) and therefore should not be limiting in ovariectomised animals. However ovariectomised pregnant gilts exhibited impaired mammogenesis when pregnancy was maintained by exogenous progesterone suggesting that increasing estrogen concentration is not the only requirement for mammary growth (Ellicott and Dziuk, 1973; Buttle, 1988). Normal mammary development occurred in gilts which retained their ovaries but had their corpora lutea excised between d 60 to 65 of gestation (Buttle, 1988). Ellicott and Dziuk (1973) and Buttle (1988) therefore propose an ovarian factor necessary for mammogenesis in pigs and have suggested relaxin as a candidate.

1.2.3.2 Lactogenesis

Lactogenesis is the initiation of lactation and it is generally defined as a two stage process. In lactogenesis I the organelles associated with milk synthesis appear in mammary epithelial cells and there is gradual accumulation of pre-colostrum in the alveolar lumina. Lactogenesis II is the onset of copious milk secretion; it can be considered to be the period from the start of normal milk production until a steady production level is reached (Fleet *et al.*, 1975; Tucker, 1988).

Changes in intracellular mammary epithelial cell structure during lactogenesis I and lactogenesis II have been described by Kensinger *et al.* (1986) and are essentially similar to those occurring in other species (Cowie *et al.*, 1980). By d 105 of gestation the Golgi apparatus has taken up a supranuclear position, there is an increasing number of polysomes and there are increasingly large lipid droplets in the cytoplasm. The alveolar lumina is larger than at 90 d and Kensinger *et al.* (1986) reported that it contained a greater number of lipid droplets, however this observation was not duplicated by Cross *et al.* (1958) who observed large variations in the time of appearance of lipid droplets in the alveolar lumina with the earliest occurrence being 5 d before farrowing.

By d 112 of gestation there were many lipid droplets in the cytoplasm but few in the lumina (Kensinger *et al.*, 1986) supporting Cross *et al.*'s (1958) observation. Rough endoplasmic reticulum (RER) had become located in the basal region of the cell and there appeared to be some secretion of protein suggesting that the Golgi apparatus was starting to function. Microvilli had elongated. Six hours after parturition microvilli had elongated further, Golgi bodies (GB) appeared functional and had increased in size and lipid droplets were concentrated in the apical regions of the cell. Secretions had the appearance of normal milk. By d 4 of lactation ultrastructural differentiation appeared to be complete with mammary cells showing the typical polarized arrangement, RER basal with supranuclear GB, of lactating mammary tissue. The number of apical lipid droplets was lower than on d 112 and the alveolar lumina were larger.

Kensinger *et al.* (1986) concluded from their histological data that stage I of lactogenesis occurs between d 90 and 105 of pregnancy with stage II occurring just prior to parturition. However, as there was further tissue differentiation between parturition and d 4 of lactation, lactogenesis II is clearly not complete at time of parturition. There was considerable variation in time of onset of lactogenesis I between individual sows (Cross *et al.*, 1958).

1.2.3.3 Control of lactogenesis

In general the minimal hormone requirements for lactogenesis II are increased prolactin, glucocorticoid and estradiol-17β secretion combined with a decline in plasma progesterone concentration (Tucker, 1988). However, insulin must be present in *in vitro* mammary cultures before other lactogenic hormones can have an effect (Denamur, 1971). This has been demonstrated in the sow by Jerry *et al.* (1989) who incubated mammary explants taken from gilts between d 91 and 105 of gestation with various combinations of insulin, cortisol and prolactin. They measured lactogenesis as an increase in glucose oxidation and glucose incorporation into fatty acids and demonstrated a significant response to increasing insulin concentrations from 10 to 100 ng/mL when cortisol and prolactin concentrations were held constant. Prolactin produced an even greater response when its concentration was changed in the presence of constant levels of cortisol and insulin. They concluded that the lactogenic complex required by the sow includes insulin, prolactin,and cortisol, however, as they did not investigate changing the concentration of cortisol in the incubation medium this conclusion may be premature. The work of Martin *et al.* (1978) indicated that cortisol does not have an active role in lactogenesis II in the sow.

In the sow, the two most important hormones determining onset of lactogenesis II are prolactin and progesterone. If the parturient prolactin peak is suppressed lactogenesis does not occur (Whitacre and Threlfall, 1981; Taverne *et al.*, 1982). This is discussed in greater detail in Chapter 4. In rodents it has been conclusively demonstrated that progesterone withdrawal is the trigger for lactogenesis II (Kuhn, 1969; Houdebine and Gaye, 1975; Djiane and Durand, 1977). Whilst progesterone withdrawal is also known to be important in other mammals the progesterone block to lactogenesis can be overcome. For example, in rabbits injection of prolactin during pregnancy immediately stimulates the onset of lactation (Houdebine *et al.*, 1985). In cattle, milking or suckling prior to parturition can induce lactation in some of the animals even though progesterone concentrations are still high (Pennington and Malven, 1985; Malven *et al.*, 1987). In the sow, Taverne *et al.* (1982) demonstrated that progesterone decline and prolactin increase do not initiate each other although they do occur during the same time period. Both are caused by an increase in prostaglandin $F_2\alpha$ concentration. Suppression of prostaglandin during late gestation postponed both parturition and lactogenesis. Suppression of the prolactin peak did not affect time of progesterone decline or of parturition, whereas artificially maintaining progesterone levels at their prefarrowing values prevented parturition but not the prolactin peak.

There is some discrepancy in the literature regarding the measurement of the onset of lactogenesis. Many workers have measured it as the earliest time that fluid can first be expressed from the teats (Nellor, 1975; Sherwood *et al.*, 1978; Nara and First, 1981). However, Willcox *et al.*, (1983) rejected this measurement because they found no relationship between the time when fluid could first be expressed and changes in composition of mammary secretions. Instead they suggested that the timing of the parturient increase in plasma lactose concentration gives a much better estimate of timing of lactogenesis. There is a close correlation between changes in lactose concentration in colostrum and those in peripheral plasma (Hartmann *et al.*, 1984). A number of workers have found a close negative relationship between plasma progesterone concentration and lactose concentration in plasma or mammary secretion during the parturient period (Martin *et al.*, 1978; Willcox *et al.*, 1983; Holmes and Hartmann, 1993) which supports the theory that progesterone withdrawal is required before lactogenesis can occur in the pig. Gooneratne *et al.* (1979) delayed parturition by approximately 1 d with exogenous progesterone and found no effect on timing of the initiation of lactation relative to time of farrowing. Taken together these data strongly support progesterone withdrawal as the lactogenic trigger in the sow.

Liptrap (1980) found that sows which had elevated progesterone concentrations following parturition had significantly lower milk production as measured by litter weight gains. De Passillé *et al.* (1993) found that there was a negative correlation between sow plasma progesterone levels during the 48 hours after farrowing and piglet growth rates in the first three days of life. Therefore rate of decline of progesterone following farrowing may be critical for the successful establishment of lactation. A clearer understanding of factors affecting progesterone concentration and its rate of decline during the parturient period might enable us to control the timing of lactogenesis and hence, perhaps reduce the incidence of postpartum agalactia. Level of feeding and animal fatness are two factors which can be manipulated fairly easily and which may significantly alter progesterone metabolism. Chapter 2 describes an experiment in which the impact of these two variables upon progesterone kinetics were measured using a progesterone-infused, ovariectomised gilt model. Chapter 2 also includes further discussion of the effects of feed intake and body fatness on clearance rate and half-life of progesterone.

Another requirement for successful lactogenesis is removal of colostrum from the mammary gland (Hartmann *et al.*, 1995a,b) Atwood and Hartmann (1993) demonstrated that fat concentration in colostrum increases rapidly in respose to piglets suckling from the gland. In contrast, unsuckled glands did not show this response. They suggest that this progressive increase in colostral fat concentration may be due to a teat-alveoli gradient. Histological studies have shown that lipid droplets accumulate in the alveoli during lactogenesis I (Kensinger *et al.*, 1986). Atwood and Hartmann (1993) postulate that the act of suckling starts to draw these lipid droplets down into the teat as colostrum is removed. The increase in fat concentration is maintained as the piglets continue to suckle and therefore there must be a corresponding increase in fat synthesis by the mammary gland. Work in the rat has demonstrated that initiation of fatty acid synthesis by mammary gland requires both progesterone withdrawal and removal of milk from the gland by sucking (Martyn and Hansen, 1980, 1981). Presumably a similar control operates in pigs. Colostrum removal by suckling piglets also resulted in increased lactose concentration of colostrum (Atwood *et al.*, 1995).

Hartmann *et al.* (1995a,b) postulated that an autocrine mechanism exists which controls induction of lactation in individual glands in response to suckling and that this ensures glands do not start producing milk until piglets are available to drink from them.

The dramatic changes in hormone concentrations occuring during the periparturient period cause major changes in the corresponding receptor populations of various target tissues. Collier *et al.* (1984) suggested that these hormone-induced changes in receptor populations determine the success of the subsequent lactation. The key changes in receptor populations during the periparturient period include the disappearance of progesterone receptors from mammary tissue (Haslem and Shyamala, 1979) so that lactating mammary tissue is completely insensitive to progesterone stimulation. Insulin receptors increase in mammary tissue (O'Keefe and Cuatrecasas, 1974) and decrease in adipose tissue (Flint *et al.*, 1979) and this is discussed in greater detail under "Endocrine control of lactation". Prolactin binding increases from late pregnancy through into lactation in the rabbit (Djiane *et al.*, 1977). In sows, prolactin binding increased two fold between 11 d before and 4 d after farrowing and then continued to increase linearly through to d 42 of lactation (Plaut *et al.*, 1989). Insulin-like growth factor-1 (IGF-1) receptors increase in the mammary gland during lactogenesis (DeHoff *et al.*, 1988), although IGF-1 concentration does not appear to affect milk production (Flint,1995). The experiment described in Chapter 5 investigated the abundance of messenger ribonucleic acid (mRNA) for progesterone and prolactin receptors during the periparturient period to investigate transcriptional control of receptor levels.

1.2.4 Control of lactation

1.2.4.1 Milk removal

Continued removal of milk from the mammary gland is essential for the maintenance of mammary secretion (Hartmann *et al.*, 1995ab). Intensity of suckling is an important determinant of milk production which stimulates lactation in two ways (Tucker, 1988). Firstly by removing autocrine inhibitory factors such as Feedback Inhibitor of Lactation (FIL, Wilde *et al.* 1995) and secondly by stimulating release of galactopoietic hormones such as prolactin (Threlfall *et al.*, 1974; Bevers *et al.*, 1978; Dusza and Krzymowska, 1981). There is a strong linear relationship between litter size and total milk production as litter size increases from four to fourteen in number (Auldist and King, 1995), largely due to an increased number of functional mammary glands. Milk production does vary between glands, however, in response to the stimulus provided by the piglet sucking that particular gland. Heavier, more vigorous piglets stimulate greater milk production than lighter piglets (Hartman *et al.*, 1962; Hemsworth *et al.*,1976) so that birthweight is a primary determinant of piglet milk intake and preweaning growth rate (Auldist and King, 1995). Sow milk production is also increased by increased frequency of suckling (Auldist and King, 1995). In this positive feedback system, sow milk production determines piglet growth rate (Noblet and Etienne, 1989) and piglet demand determines sow milk production (Van der Steen and de Groot, 1992). Nevertheless Boyd *et al.* (1995) suggest that sow milk production is limiting piglet growth rate from d 8 of lactation and that this results in lost potential preweaning growth of more than 40% when compared to artificially reared piglets. Sows often produce well below their potential, as has been shown when sows were stimulated by rearing two litters simultaneously (Sauber *et al.* 1994) or by placing older piglets onto a newly farrowed sow (Auldist and King, 1995). Sow milk production can be stimulated by improving nutrition to the sow or maybe by manipulating the endocrine control of lactation.

1.2.4.2 Nutritional control of lactation

There have been many excellent reviews of nutrient requirements for lactating sows in recent years (e.g. Hughes, 1989; Cole, 1990; Aherne *et al.*, 1991; Aherne and Williams, 1992; Einarsson and Rojkittikhun, 1993; den Hartog *et al.*, 1994; Pettigrew, 1995). It is widely recognised that a sow's feed intake during lactation frequently fails to meet her nutrient requirements so that she uses her body reserves to support lactation and this can adversely affect milk production and consequently litter weight gain (King and Dunkin, 1985; Mullan and Williams, 1989; Yang *et al.*, 1989). However, during the periparturient period (weeks 1 and 2 of lactation) the sow is remarkably tolerant of nutrient shortage, at least as far as lactation is concerned. When sows received different feed intakes of the same diet during the first two weeks of lactation there was invariably no effect on piglet weight gain over this period (King and Dunkin, 1985; Noblet and Etienne, 1986; Lythgoe, 1987; Yang *et al.*, 1989; Black *et al*, 1993;

Prunier *et al.*, 1993). However, piglet weight gains in early lactation were influenced by gestation feeding level and, or, sow body weight and fatness at farrowing (Mullan and Williams, 1989; Yang *et al.*, 1989), although in the experiment of Mullan and Williams (1989), this was due to variations in piglet birthweight.

Prolactin and insulin are believed to be the main galactopoietic hormones in sows (Collier *et al.*, 1984; Forsyth, 1986; Vernon, 1989) and plasma levels of both of these hormones should be increased by an increased level of feeding (Rojkittikhun *et al.*, 1993; Weldon *et al.*, 1994) so that milk production in high intake sows should be stimulated to a greater extent than that in low intake sows. However, as noted above, low feed intake frequently does not result in reduced nutrient output even though milk composition may have changed as a result of the differing metabolic states of the sows (Noblet and Etienne, 1986). The relationship between feed intake, plasma prolactin and insulin concentrations, and milk output in the first two weeks of lactation has been examined in Chapter 4 of this thesis. It is possible that receptors for insulin and prolactin are down-regulated in mammary tissue of high feed intake sows so that the perceived stimulation is similar to that of sows with low feed intake. In Chapter 5 this question has been partially addressed by examining gene expression of prolactin receptors in mammary tissue from sows on widely different feed intakes, sampled on d 14 of lactation.

Recent work has demonstrated that sows will respond to higher dietary concentrations of lysine and valine than had previously been fed (Tokach, 1991;

Richert, 1994, 1995). Unfortunately the data presented does not describe whether increased piglet growth rate is evident by the end of the second week of lactation. However the greatest response to increases in dietary lysine and valine concentrations was recorded in high producing sows (Richert, 1995) with no reponse in low producing sows. Therefore, it is likely that effects will not be significant by the end of the second week of lactation as sows will not reach peak lactation until the end of wk 3 (Noblet and Etienne, 1986). It seems that the sow is able to buffer the effects of inadequate nutrition during the first 2 weeks of lactation, probably because her capacity to produce milk is not being stretched to its limit at this time.

1.2.4.3 Endocrine control of lactation

In the sow prolactin and insulin play a major role in the maintenance of milk secretion (Hartmann *et al.*, 1995ab). Prolactin is the dominant hormone of lactation (Flint, 1995). In the lactating mammary gland it regulates synthesis of milk proteins (Houdebine *et al.*, 1985), synthesis of milk-specific fatty acids and the uptake of fatty acids (Vonderhaar, 1987). It is also involved in osmoregulation and induces synthesis and secretion of components of the secretory immune system (Vonderhaar, 1987). In established lactation suppression of prolactin secretion causes a decline in milk production (Boyd *et al.*,1995; Farmer, personal communication), and in lactose production (Flint, 1995). This is discussed in greater detail in Chapter 4.

In the rat at parturition the numbers of insulin receptors on the surface of mammary epithelial cells increase (O'Keefe and Cuatrecasas, 1974) whilst the numbers on adipocytes decrease (Flint *et al.*, 1979). This phenomenon, known as peripheral insulin resistance, results in increased sensitivity of mammary tissue to insulin, even at low blood insulin concentrations, in conjunction with reduced adipose sensitivity to insulin so that lipogenesis in adipose tissue is reduced whilst lipolysis is increased and energy substrates are redirected to the mammary gland for milk production (Vernon, 1989). In ruminants a similar repartitioning of nutrients occurs in lactating animals but in this case insulin binding to adipose tissue is unaffected and the reduced responsiveness of adipose tissue to insulin during lactation is thought to be due to a post-receptor defect (Vernon *et al.*, 1981). In sheep glucose uptake in the hindlimb was reduced during lactation (Vernon *et al.*, 1990). Insulin resistance in non-mammary peripheral tissues and liver during lactation makes glucose more available for milk production. As in other species the sow mammary gland does not require insulin to take up glucose (Hartmann *et al.* 1995) and therefore glucose utilization proceeds regardless of vagaries in insulin concentration induced by feeding pattern (Bauman and Elliot, 1983). This is particularly important because lactose, which is derived from glucose, is the main osmolite in milk and therefore the determinant of milk yield (Faulkner and Peaker, 1987). Glucose uptake is determined by its plasma concentration, mammary blood flow (Faulkner and Peaker, 1987) and may also be regulated by the concentration of glucose transporters in the mammary epithelial cells.

In ruminants growth hormone (GH) is the most important regulator of milk synthesis (Bauman and Currie, 1980; Bauman and Elliot, 1983) but in pigs its role is less clear. Attempts to stimulate milk production by exogenous GH administration have met with mixed results. Harkins *et al.* (1989) did acheive a 22% increase in milk yield in lactating sows by daily administration of porcine somatotropin from d 12 to d 28 of lactation, however other attempts to stimulate lactation with porcine somatotropin have been unsuccessful (Cromwell *et al.*, 1992; Toner *et al.*, 1991). Armstrong *et al.* (1994) immunised cyclic gilts against growth hormone-releasing factor (GRF) which resulted in reduced circulating GH and IGF-1 concentrations and increased thyroxine. During the subsequent lactation, milk consumption of piglets on d 20 was decreased compared to controls but there was no corresponding difference in piglet growth rate perhaps because of the increased concentration of lipid in the milk of the treated sows. Likewise attempts to stimulate milk production by administering GRF to lactating sows have been unsuccessful (Dubreil *et al.*, 1990; Farmer *et al.*, 1992, 1996). Although Farmer *et al.* (1996) did observe increased piglet growth rates for GRF treated sows on d 14 of lactation, this was no longer significant by d 21 perhaps because of the lower feed intake of the GRF supplemented sows. Therefore GH does not appear to have an important role in the control of sow milk production.

1.2.5 The importance of glucose transporters in mammary tissue to the periparturient sow

Glucose is a primary fuel for mammary metabolism particularly during lactation when it is the sole precursor for lactose synthesis and also a major precursor for *de novo* fatty acid synthesis where it is required to supply NADPH and glycerol and may also be used in the synthesis of fatty acids (Faulkner and Peaker, 1987). A number of glucose transporters have been identified in mammalian tissue including a sodium-dependent active transporter which is found in the brush-border membranes of intestinal and kidney epithelial cells and a family of facilitative glucose transporters which are expressed in a highly controlled tissue specific manner thus playing an important part in whole-body glucose homeostasis (Burant *et al.*, 1991). Six functional facilitative glucose transporters have so far been identified, and these have been comprehensively reviewed by Burant *et al.* (1991) and Gould and Holman (1993). The main glucose transporter of interest in mammary gland is the erythrocyte-type glucose transporter known as GLUT1. This is the only glucose transporter expressed by lactating mammary gland (Burnol *et al.*, 1990; Madon *et al.*, 1990; Zhao *et al.*, 1993; Camps *et al.*, 1994). Although the insulin-responsive glucose transporter GLUT4 has also been detected in non lactating mammary tissue it is thought to be associated with adipocytes not mammary epithelial cells (Burnol *et al.*, 1990). Expression of GLUT4 in mammary tissue decreased progressively throughout gestation and was not detected during lactation (Burnol *et al.*, 1990; Camps *et al.*, 1994).

GLUT1 is widely distributed in the body and is frequently found in the cells of blood-tissue barriers. This transporter has asymmetric transport kinetics with the efflux having about fourfold greater K_m and V_{max} than the influx (Carruthers, 1990). It should therefore provide a one way transport system and be able to transport glucose into cells when extracellular glucose is low and the intracellular demand for glucose is high (Gould and Holman, 1993).

GLUT1 transporter in mammary gland is not regulated by insulin (Fawcett *et al.*, 1991) and therefore glucose uptake by mammary tissue should be directly related to the number of glucose transporters. In Chapter 5 gene expression of GLUT1 in the mammary gland of sows receiving different feed intakes was related to milk yield and composition.

1.2.6 Preparing for the next pregnancy

1.2.6.1 Uterine Involution

Uterine involution is a complex process involving an overall reduction in uterine size associated with contraction and atrophy of muscle cells, elimination of lochia, reduction in blood supply and regeneration of the endometrium (Graves *et al.*, 1967; Slama *et al.*, 1991). Limited work has been reported on uterine involution in the sow. Estimates of when involution is complete vary with the parameter being measured. In terms of uterine size involution is considered to be complete by between d 21 and d 28 postpartum (Palmer *et al.*, 1965a; Svajgr *al.*, 1974). The uterine epithelium degenerates over the first few days postpartum with regeneration commencing from around d 7. Complete regeneration of the epithelium probably occurs soon after d 14 (Rumjancev, 1954 cited by Palmer *et al.*, 1965b; Palmer *et al.*, 1965b). Rate of uterine involution can vary between breeds (Graves *et al.*, 1967) and is greater in sows that are suckling than in weaned sows (Graves *et al.*, 1967). Metritis or pyometra prolong involution in cows (Slama *et al.*, 1991) and probably have the same effect in sows although this has not been reported. Feeding level in lactation does not appear to affect rate of uterine involution. In a trial in which sows were fed either *ad libitum* or 1.25 times maintenance for the first two weeks of lactation there was no difference in uterine weight at 14 d postpartum (Miller *et al.*, unpublished data).

Complete uterine involution may not be necessary for the sow to start another pregnancy. Svajgr *et al.* (1974) weaned sows after a two day lactation and bred them an average of ten days later so that embryos would be entering the uterus on d 15. The majority of sows were able to establish and maintain a pregnancy even though embryo survival was reduced (54.3%, versus 70.7% for sows weaned after 13 d of lactation and 79.5% for sows weaned after 39 days). Reduced embryo survival may be due to incomplete uterine involution and therefore failure to establish optimum uterine conditions for the implanting embryos, however it may equally be due to a poorer quality of embryo resulting from inadequate follicular development prior to ovulation.

1.2.6.2 Nutrition in the periparturient period and subsequent reproductive performance

Following farrowing there are a number of large follicles present in the ovary presumably due to the high steroid hormone levels and luteinising hormone (LH) pulsatility in the periparturient sow but these become atretic leaving only small follicles by the end of the first week of lactation (Palmer *et al.*,1965b). In response to the suckling stimulus, LH declines rapidly by d 3 (de Rensis *et al*, 1993) and thereafter remains at low levels until weaning. Likewise follicle development is constrained over this period with a reduction in the proportion of atretic follicles and a gradual increase in follicular size as lactation progresses followed by a dramatic increase immediately following weaning (see review by Britt *et al.*, 1985).

It is well established that low feed intakes in lactation are associated with reduced subsequent reproductive performance especially in primiparous sows. This may be variously expressed as one, or a combination of, an extended weaning to mating interval (WEI, King and Williams, 1984, Baidoo *et al.*, 1992), decreased ovulation rate (Foxcroft *et al.*, 1995), reduced embryo survival and smaller subsequent litter size (Hughes, 1989). There are many good reviews describing the relationships between sow nutrition and optimum reproductive performance (e.g. Aherne and Kirkwood, 1985; Hughes, 1989; Cole, 1990; Aherne and Williams, 1992; Whittemore, 1995). However, the importance of pattern of feeding in lactation is still unclear. Workers from the University of Minnesota have suggested that nutritional deprivation in early lactation can affect the fertility of the sow after weaning (Tokach *et al.*, 1992, Koketsu, 1994). In a retrospective analysis of his data, Tokach (1991) found that insulin concentration on d 7 of lactation was significantly correlated with LH pulsatility on d 14, 21, and 28. Sows showing a prolonged weaning to estrus interval (> 15 d) had significantly lower serum insulin on d 7 of lactation than those which returned to estrus within 9 days. Koketsu *et al.* (1994) have suggested that reducing feed intake in the first or second week of lactation has a greater detrimental effect on subsequent sow fertility than reducing feed intake in later lactation. It is also possible that follicles ovulating after weaning may first emerge from the primordial pool of follicles during late gestation. If true this could be of great practical importance, particularly to the very early weaned sow, as many conventionally managed sows become catabolic in late gestation. The earlier work of Clark *et al.* (1972) and Dailey *et al.* (1972) suggested that increasing feed intake increased ovulation rate by increasing the the size of the follicle pool available for recruitment. As insulin reduces atresia of follicles and increases ovulation rate (Cox *et al.*, 1987, Matamoros *et al.*, 1990, 1991), high insulin in early lactation may allow more follicles to remain within the recruited pool ultimately providing a greater estrogenic stimulus at weaning. Recruitable follicles require 19 d of maturation from the antral stage to ovulation (Morbeck *et al.*, 1992) and therefore may be affected by nutritional status of the sow in early lactation. Extensive studies of the endocrine mechanisms mediating the effects of nutrient intake on ovarian function in the prepubertal gilt have been conducted at the Universities of Alberta and Nottingham (Booth, 1990). These indicate that the dynamic changes in gonadotrophin secretion immediately before the recruitment of follicles into the follicular phase are a critical determinant of subsequent fertility. Prepubertal gilts which were switched from maintenance level feeding to *ad libitum* feeding showed an immediate response in LH pulsatility and plasma glucose and insulin concentrations. In a separate experiment these changes were reflected in superior follicular development and greater LH and FSH peak responses to an LHRH challenge after 13 d of *ad libitum* feeding compared to gilts which had been maintenance fed over the same period. These immediate effects of the dynamic metabolic state of the gilt clearly involve both central (Cosgrove *et al.*, 1993) and local ovarian (Cosgrove *et al.*, 1992; Charlton *et al.*, 1993) effects.

Whilst nutrition in early lactation, and even perhaps in late gestation, can apparently affect subsequent reproductive performance, the work of Zak *et al.* (reported by Aherne *et al.*, 1995) and the more recent work of Koketsu *et al.* (1996) indicate that the effects of nutritional insufficiency become increasingly profound the closer they are to weaning. Zak *et al.* used primiparous sows with a standardised litter size of six piglets to investigate the effects of three patterns of feeding during lactation on subsequent reproductive performance. Sows were fed 1) to appetite for one hour three times per day throughout lactation, or 2) to appetite from farrowing until d 21 of lactation and then restricted to 50% of the to appetite intake for the remainder of lactation, or 3) sows were restricted to 50% of to appetite intake from farrowing to d 21 and then fed to appetite until d 28 of lactation. Pattern of feeding in lactation did not affect the pattern of LH secretion 12 hours post weaning, but both restricted groups had a longer WEI and lower ovulation rate than unrestricted sows. In addition there was a reduction in embryo survival in sows which were restricted during the last week of lactation so that this group had the lowest fertility of all the treatments. This experiment showed that the detrimental effects of low feed intake for three out of four weeks of lactation could be partially offset by adequate feeding in the week before weaning. In contrast, restricted feeding for just one week immediately prior to weaning even after adequate feeding for the whole of the rest of lactation was even more detrimental to subsequent reproductive performance. Likewise when Koketsu *et al.* (1996) investigated the effects of reducing feed intake in week 1 or week 2 or week 3 of a 21 d lactation on WEI, they found that WEI was longer in sows which had been restricted during weeks 2 or 3. There was no significant effect of reducing feed intake in week 1 although WEI was numerically longer than that of controls which had been full fed throughout. Unfortunately they did not measure ovulation rate, embryo survival or subsequent litter size. They did observe that LH pulsatility was significantly reduced by low feed intake but only during the period of low feed intake.

Reconciling these apparently conflicting results may provide a complete basis for explaining the mechanisms mediating the effects of nutrition during lactation on post weaning fertility. It has been noted already that one potential mechanism by which nutrition may affect ovulation rate is by changing the size of the follicle pool available for recruitment (Clark *et al.*,1972; Dailey *et al.*, 1972). The sensitivity of the ovary to gonadotrophin stimulation is affected by the metabolic status of the sow. A large body of *in vitro* data indicates that intermediate metabolites and the hormones controlling intermediary metabolism can affect follicular function in the pig (Hammond *et al.*, 1993) and some of these effects may be mediated through classic endocrine mechanisms. Treatment with exogenous hormones therefore has the potential to imitate these effects thus explaining the beneficial responses to insulin administration obtained by Cox *et al.* (1987) and Matamoros *et al.* (1991). Nutritionally-mediated changes in metabolic state are also expected to cause changes in autocrine and paracrine growth factor activity in the ovary. Preliminary *in vivo* data of Charlton *et al.* (1993) supported this suggestion. The reported associations between nutritionally-induced differences in insulin secretion during lactation and subsequent fertility may therefore involve direct ovarian effects, as was suggested by the work of Cox *et al.* (1987), as well as indirect central effects on LH secretion. As all stages of follicular development may be sensitive to such changes, the "imprinting" of follicles in early lactation may have lasting consequences for the ultimate size of the recruitable pool of follicles at weaning. Furthermore, as the quality, as well as the quantity, of follicles appears to be important for normal oocyte maturation and early embryonic development (Ding and Foxcroft, 1994), the imprinting of follicles due to different metabolic states may have consequences for embryonic survival.

In conclusion, therefore, it is likely that nutritional insufficiency during any period of lactation, and even possibly in late gestation, can adversely affect postweaning reproductive performance. However, the work of Zak *et al.* (reported by Aherne *et al.*, 1995) and Koketsu *et al.* (1996) suggests that the more immediate the nutritional inadequacy is to weaning, the greater the insult. Chapter 4 describes an experiment in which follicular development and LH pulsatility were measured at the end of the second week of lactation in primiparous sows which had received restricted or *ad libitum* feed intake.

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CHAPTER 2

The Effect of Feed Intake and Body Fatness on Metabolic Clearance Rate of Progesterone in Ovariectomised Gilts

2.1 Introduction

Preweaning mortality can exceed 20% of liveborn piglets (English and Wilkinson, 1982). More than 70% of these mortalities occur within the first three days of life and the events which take place at this time are frequently responsible for subsequent mortalities as well (Dyck and Swierstra, 1987). Piglets which die are characterised by low energy intakes resulting in low growth rates. De Passillé *et al.* (1989) observed that multiple deaths within litters were correlated with poor weight gains of surviving piglets. They suggested that some sows may have difficulty establishing lactation (lactogenesis). Progesterone has been shown to inhibit lactogenesis in some species (Tucker, 1988). It has been demonstrated convincingly in rats that progesterone withdrawal is the trigger for the onset of copious milk secretion (Kuhn, 1969) and it is thought that the same mechanism operates in the sow (Hartmann and Holmes, 1989). De Passillé *et al.* (1993) found that there was a negative correlation between sow plasma progesterone levels over the 48 h after farrowing and piglet growth rates in the first three days of life. This suggests that the rate of decline of progesterone after farrowing may be crucial to the satisfactory establishment of early lactation.

It is important to understand the factors which control progesterone kinetics so that sows can be managed in a way which will optimise progesterone levels during and after farrowing to allow effective establishment of lactation. Level of feeding and animal fatness are two factors which can be manipulated fairly easily and which may significantly alter progesterone metabolism.

Cumming *et al.* (1975) were the first to notice an inverse relationship between plane of nutrition and peripheral plasma progesterone concentration in sheep. It was subsequently found that this inverse relationship occurred whether progesterone was from endogenous or exogenous sources, clearly indicating that high feeding levels resulted in increased metabolism of progesterone and not in reduced secretion rate (Parr *et al.*, 1982). When the feed intake of sheep was increased from half maintenance to twice maintenance, metabolic clearance rate (MCR) of progesterone increased from 118 to 217 mL/min/kg body weight (BW) ($P < 0.05$, Parr *et al.* 1993a). The mean rate of blood flow in the portal vein increased from 2.2 to 3.5 L/min ($P < 0.05$) in response to the same increase in feed intake (Parr *et al.*, 1993b). The liveweight of the sheep used in the latter trial was not provided but, assuming a value similar to that described in the earlier experiment (Parr *et al.* 1993a) of about 45 kg, portal blood flow rates would be from 49 to 71 mL/min/kg BW, respectively. Taking into account blood flow through the hepatic artery which is 26% of total liver blood flow postprandially (Katz and Bergman, 1969, cited by Richardson and Withrington, 1981), total liver blood flow rates would be approximately 62 and 90 mL/min/kg BW respectively. It is immediately apparent that MCR of progesterone greatly exceeds the volume of blood passing through the liver and indeed it is generally considered that splanchnic extraction and nonsplanchnic extraction are approximately equal (Baird *et al.* 1969). However splanchnic extraction is more efficient since the liver is able to extract more than 90% of progesterone from blood passing through it (Bedford *et al.* 1974; Parr *et al.*, 1993b) and therefore increasing liver blood flow will increase MCR. Parr *et al.* (1993b) concluded that the increase in splanchnic blood flow in response to increased feeding level explained the corresponding increase in MCR of progesterone. However, comparison of MCR and blood flow data indicates that at half maintenance feeding clearance by the liver represents 52.2% of total clearance, whereas at twice maintenance it represents only 41.24% (these calculations assume 100% progesterone extraction in one pass through the liver). This suggests that the increase in liver blood flow rate is not the only effect of increasing feed intake on MCR of progesterone.

Similar observations to those made with sheep have been made in pigs. It was noted that gilts fed low intakes (1.5 kg/d) had significantly higher plasma progesterone concentrations in early pregnancy than gilts fed high intakes (3.0 kg/d, $P < 0.01$, Dyck *et al.*, 1980). It has also been shown consistently that when pigs are fed following a period of fasting there is a dramatic increase in portal and hepatic blood flow rates (Braude *et al.*, 1970; Anderson, 1974; Farrell *et al.*, 1974; White *et al.*,

1974) however, it was not known whether hepatic blood flow would respond similarly to different levels of feed intake, with low feed intakes resulting in lower blood flows than high feed intakes. To test whether feed intake had the same effect on MCR and blood flow rate in pigs as it did in sheep, Prime and Symonds (1993) investigated the effect of feeding 1 versus 3 kg per d to ovariectomised gilts between 65 and 110 kg liveweight. Their results did show a significant increase of 47% in MCR of progesterone between the low and the high levels of intake, from 41.0 to 60.3 mL/min/kg BW, and this was significantly correlated to portal blood flow rate which increased by 45%. Unfortunately their blood flow rates were unrealistically low due to functional problems with their ultrasonic probe detection system and therefore the measured increase must be regarded with skepticism.

Splanchnic extraction of progesterone, primarily by the liver, has been estimated to account for between 27% (Bedford *et al.*, 1974, in sheep) and 70% (Little *et al.*, 1966, in humans) of total progesterone clearance. The liver is considered to be the main organ of progesterone metabolism because it is able to metabolise all free and albumin bound progesterone passing through it, whilst other tissues are less efficient. The mixed function oxidases (MFO), which are cytochrome P450 enzymes, metabolise progesterone in the liver. In addition to increasing blood flow through the liver, greater feed intake may also stimulate an increase in the number of hepatocytes (liver size) and, or up-regulation of the concentration of hepatic MFO. This has been demonstrated in female rats which increase hepatic cytochrome P450 enzyme levels in response to increased feed intake (Hashmi *et al.*, 1986) and in response to increasing quality and quantity of dietary protein (Clinton *et al.*, 1977; Bidlack, 1986). Level of fatness of sows may also affect hepatic MFO levels since it has been observed that obese mice had significantly lower hepatic cytochrome P450 enzyme levels than their non-obese littermates, however when the obese mice were feed-restricted until they were of similar fatness to their non-obese littermates their cytochrome P450 levels returned to normal (Dorsey and Smith, 1981).

In studying the dynamics of progesterone metabolism it is important to remember that progesterone is lipophilic. Hillbrand and Elsaesser (1983) found that the concentration of progesterone in adipose tissue could be more than 200 times greater than that in plasma. As sow fatness can vary greatly between individuals, it is necessary to understand how adipose mass affects rate of decline of progesterone around farrowing. When plasma progesterone is in equilibrium with adipose progesterone fatter sows will have a greater total body store of progesterone than leaner sows. It is therefore likely that such sows will take longer to clear progesterone from the body when its secretion ends, such as at farrowing or at the end of the luteal phase of the oestrous cycle. This was not confirmed by Hillbrand and Elsaesser (1983). They reported that, after ovariectomy twelve or thirteen days after standing oestrus or following intra muscular progesterone injection, gilts showed no clear effect of the fat to lean ratio on changes in backfat or plasma progesterone concentrations during the oestrous cycle. They did note that fatter gilts took longer to reach their maximum plasma progesterone concentration during the oestrus cycle or following intra muscular progesterone injection.

The aim of the present experiment was to confirm the hypothesis of Prime and Symonds (1993), that increased feed intake increases MCR of progesterone. In addition the effects of fatness on MCR, and of fatness and feeding level on the rate of decline of plasma progesterone following withdrawal of the source of progesterone were studied. Ovariectomised pubertal gilts were used to model progesterone kinetics in the sow during late gestation and early lactation since it has been shown that immature gilts (less than 95 kg) have relatively higher MCRs of steroid hormones (Elsaesser *et al.*, 1982; Christenson *et al.*, 1985). All gilts were ovariectomised to remove their primary endogenous source of progesterone, namely the corpora lutea of the ovary. Some progesterone is also produced by the adrenal gland but, under normal circumstances, this is a negligible amount.

2.1.1 Hypotheses

On the basis of the literature cited, the present experiment was conducted to test the following hypotheses

- increasing feed intake will increase both MCR and rate of decline of progesterone.
- Fatter gilts will have similar MCR and slower rates of decline of progesterone than thinner gilts.
- Liver size, MFO levels and activity will be higher in high feed intake animals than in low feed intake animals.

2.2 Materials and Methods

2.2.1 Animals

Forty Camborough x Canabrid gilts (Pig Improvement (Canada) Ltd, Acme, AB, Canada), comprising 10 groups of four litter sisters, (ten groups of five to six litter sisters were selected initially to ensure four litter mates per litter) were used in this experiment, with one gilt from each litter being randomly allocated to each of the four treatments described below. The remaining ten gilts were retained to act as non-infused, non-ovariectomised controls.

2.2.2 Experimental treatments and animal management

There were two feeding levels and gilts had been reared to achieve similar protein masses but different fatness levels at the time of ovariectomy. The two feed intakes were;

- Low (L) - 1.15 times maintenance, and
- High (H) - 2.30 times maintenance.

Feed intakes were calculated on the basis of a maintenance energy requirement of 460 kJ DE/kg$^{0.75}$ BW (NRC, 1988) and were fed from eight days prior to the start of the infusion until slaughter. The target weights and backfat depths for the gilts of different fatness at ovariectomy were;

- Lean (Ln) - 113 kg BW and 10 mm P2 backfat and
- Fat (F) - 124 kg BW and 20 mm P2 backfat.

When gilts reached their target size they were ovariectomised and then prepared for measurement of MCR. Difference in fatness was achieved by rearing the gilts from 30 kg liveweight along two different growth curves. F gilts received a high energy/low protein diet (see Table 2.1) *ad libitum* throughout their grower/finisher period, whilst Ln gilts received a low energy/high protein diet fed at 80% of the daily intake of the F animals, provided as a single daily feed. Since the high energy/low protein diet contained 80% of the protein content of the low energy diet this meant that, at any particular body weight, gilts consumed similar daily intakes of protein. Diets were also adjusted so that all gilts received similar amounts of minerals and vitamins. All gilts were penned and fed individually from 30 to 50 kg BW. From 50 kg onwards only the Ln gilts remained in individual pens because of housing constraints. F gilts were housed in groups of 2 or 3 with continuous access to feed. Feed consumption of F gilts was measured three times weekly. All gilts were weighed 3 times weekly to determine appropriate feed intakes. Backfat was measured weekly from 70 kg BW using an ultrasonic probe (Scanoprobe 11, Scanco, Ithaca, NY) at the last rib and 65 mm from the midline (P2).

From 100 kg onwards gilts received daily boar contact to induce puberty. Due to housing constraints the level of boar contact varied markedly between family groups. Half of the gilts were housed in the breeding room with continuous open fence line boar contact and a daily period of direct boar contact. The other half were housed in a finisher barn and allowed 30 min of open fence line contact with a boar each day. Attainment of puberty was assessed from studying the ovaries at ovariectomy.

2.2.3 Methods

When gilts reached their target weight and backfat they were ovariectomised, irrespective of known pubertal oestrus. Food was withdrawn for at least 16 h prior to surgery. Gilts were sedated with an intramuscular injection of 'Stresnil' (4% solution of azaperon or 4'fluoro-4[4-(2-pyridyl)-1-piperazinyl]-butyrophenone, Janssen Pharmaceutical Ltd., Oxford, UK; Dosage: 1 mL 'Stresnil' per 20 kg BW). Fifteen min later the animals were anaesthetized with 'Intraval' (5% solution of 5-ethyl-5-(1-methyl-butyl)-2-thiobarbituric acid, MTC Pharmaceuticals, Cambridge, Ontario, Canada; Dosage: 0.17 mL 'Intraval' per kg BW), administered via an ear vein whilst the gilts were restrained with a nose snare. Anaesthesia was maintained with a closed-circuit system of halothane and oxygen. An incision was made through the upper lumbar region of the ventral midline. The reproductive tract was located and exteriorised through the incision. In turn each ovary was carefully exposed by folding back the fimbria. The blood vessels were clamped with forceps immediately below the ovary and the ovarian artery and vein were ligated separately below the forceps. The ovary was excised above the forceps and then the forceps were removed. The reproductive tract was replaced inside the animal having been kept moist with sterile saline solution throughout. The incision was closed with interrupted sutures. After surgery each gilt was injected with a long-acting antibiotic (Liquamycin LA; oxytetracycline dosage 1 mL/10 kg body weight) and returned to an individual pen.

Following ovariectomy the gilts received a conventional finisher diet (13.5 MJ DE/kg, 15.1% CP and 0.9% lysine) at either high (2.3 x maintenance) or low (1.15 x maintenance) intake for the remainder of the experiment. Each gilt's daily ration was fed as two equal sized meals at 8 am and 4 pm.

Approximately 10 d after ovariectomy each gilt was surgically fitted with bilateral jugular catheters (silastic, Dow Corning Corporation, Michigan, USA). Anaesthesia was administered as described for the ovariectomy above. Silastic catheters were placed in both external jugular veins and exteriorised through the dorsal surface of the neck. The infusion catheter was placed in the left jugular vein to prevent the possible contamination of samples with recirculating hormone from the lymph. The thoracic lymph duct empties into the left jugular (Sisson and Grossman, 1938). The infusion catheter was at least 12 cm longer than the sampling catheter, placed in the right jugular, to reduce the chance of contamination from the infusion catheter into the sampling catheter at the confluence of the two jugulars to form the anterior vena cava (Sisson and Grossman, 1938).

2.2.4 Progesterone infusion

A preliminary experiment was conducted with two additional ovariectomised gilts to establish whether steady state for plasma progesterone could be reached more rapidly using continuous infusion or using the priming dose method of Prime (1988).

The gilts were first infused with 130 mg progesterone/day for 5 days, allowed three days without progesterone and then given intramuscular injections of approximately 130 mg progesterone suspended in peanut oil for two consecutive days followed by infusion at the same rate as before for a further three days. Plasma progesterone of the gilts was monitored throughout and it was found that steady state was reached more rapidly using the priming dose method. Therefore for the remaining gilts a similar procedure to that described by Prime (1988) was adopted. Briefly, two to three days after implanting the catheters, 5 mL blood samples were taken from each gilt before each feed for two days. Approximately 130 mg progesterone suspended in peanut oil was injected into the muscle of one hind leg of each gilt at 9 am for two consecutive days. On the third day the infusion catheter was connected to the infusion pump via polyethylene tubing (Tygon) and progesterone was infused into the gilt at the rate of approximately 5.4 mg/hour (130 mg progesterone/day) for 60 h. This prolonged period of priming and infusion was used to ensure equilibrium between plasma and backfat levels of progesterone. Hillbrand and Elsaesser (1983) have shown that changes in backfat concentrations tend to lag about two days behind changes in plasma concentration. The infusate contained 0.34 mg progesterone/mL dissolved in 20% ethanol, 80% physiological saline solution. 5 mL blood samples were taken from the sampling catheter every twenty min for the last 24 h of infusion. Rate of infusion was calculated from the hourly change in weight of the infusate bag. A backfat sample was taken from the middle of the back using a biopsy needle after approximately 32 h of infusion. After 60 h of infusion the infusion line was disconnected and any infusate remaining in the catheter was withdrawn. The infusion catheter was then flushed with saline. Blood samples were withdrawn at hourly, two hourly, four hourly, eight hourly and twenty four hourly intervals as shown in Figure 2.1. All blood samples were centrifuged at 1500 x *g* for 15 min within 10 min of collection. The plasma was collected and stored at -30°C until analysed. The gilts were slaughtered at a commercial abattoir approximately 12 h after taking the last blood sample. At slaughter the liver was removed and weighed. Samples were taken from the left lobe of the liver of each gilt (for microsomal studies) and from the backfat (for progesterone determination), these were snap frozen immediately in liquid nitrogen.

In addition, 10 non-infused, non-ovariectomised gilts comprising the remaining unselected litter sisters of the infused gilts were slaughtered at the same time as their sisters. The non-infused, non-ovariectomised gilts consisted of 4 fat and 6 lean animals which had all received high intake for 2 weeks prior to slaughter. Their livers were also removed, weighed and sampled to provide a non-infused, non-ovariectomised control group for the microsomal studies described below.

2.2.5 Progesterone analysis

Plasma samples were analysed in triplicate for progesterone by radioimmunoassay as described by Beltranena *et al.* (1991) using an antiserum raised against 4-pregnen-11α-ol-3, 20-dione hemisuccinate:BSA. Cross-reactions with this serum were: corticosterone 2.7%, pregnenolone 2%, 20α-progesterone 0.18%, estrone 0.12%, testosterone 0.06%, dihydrotestosterone 0.03%, cortisol 0.015%, cholesterol 0.003% and estradiol 0.002%.

Prior to assay 100 μL of plasma, diluted 1 in 10 with PBS gel buffer, was extracted by vigorous vortexing with 4 mL petroleum ether for 5 min. Within each assay extraction efficiency was determined by recovery of [1,2-^{3}H(N)]-progesterone and this value was used to correct the determination of progesterone within each tube. The mean recovery of tritiated progesterone was 87.9%. Standard curves ranged from 0.001 to 10 ng/tube.

Sensitivity of the assay, defined as 85% of total binding, was 0.02 ng/tube. No significant deviation from parallelism was apparent from assaying 100, 50 and 25 μL of a standard plasma pool. The intra- and inter-assay coefficients of variation for the progesterone assays were 10.0% and 23.3% respectively. To reduce the effect of inter-assay variance littermates representing each treatment were included in each assay.

2.2.6 Calculation of metabolic clearance rate

MCR was calculated for each gilt over two three hour periods; from 4:00 am to 7:00 am (preprandial period) and from 6:00 pm to 9:00 pm (postprandial period). During these periods the coefficient of variation for plasma progesterone concentration was less than 20% and therefore a mean value was calculated for each gilt. MCR was calculated from the following equation;

$$\text{MCR (mL/h)} = \frac{\text{Infusion Rate of Progesterone (ng/h)}}{\text{Plasma Progesterone Concentration(ng/mL)}}$$

2.2.7 Calculation of half life

The disappearance rate constant (k - the fraction of total hormone that disappears per unit time) of progesterone after the end of the infusion was determined by regression of the natural logarithm of plasma progesterone against time. The resulting regression fits the equation $X = Ae^{-kt}$ where k is the slope of the curve. In this calculation plasma samples taken from 1 to 72 h after the end of the infusion were used. Only regressions which were significant at $P < 0.05$ were used in the final analysis. Progesterone half life was calculated from the following equation;

$$\text{Half life (h)} = \frac{0.693}{k}$$

2.2.8 Microsomal isolation

Frozen liver samples from each of the infused gilts and from their non infused, non ovariectomised litter sisters were coarsely chopped and then homogenised (Ultra-Turrax homogenizer, Janke and Kunkel, Germany) with 5 volumes of buffer 1 (50 mM Tris-HCl, 100 mM KCl, 10 mM EDTA and 100 μM PMSF at pH 7.5). The homogenate was centrifuged at 10,000 *g* for 20 min at 4°C and the resulting supernatant was centrifuged again at 100,000 *g* for 60 min at 4°C (Lake, 1987) The supernatant was poured off and the remaining microsomal pellet was resuspended in buffer 2 (50 mM Tris-HCl, 10 mM potassium phosphate, 0.1 mM EDTA and 20%

glycerol at pH 7.4). Protein concentrations of the microsomal suspensions were determined using the BCA Protein Assay (Pierce Chemical Co., Rockford, IL).

2.2.9 Metabolism of progesterone

1 mg/mL of liver microsomes was incubated with 50 mM Tris pH 7.5, 20% glycerol, [^{3}H]-progesterone (2.0 μM, 0.12 μCi/μmol), 1.25 mM NADPH and 1.25 mM NADH. The total volume was 1.0 mL. The assay mixture, minus NADH and NADPH, was incubated at 37°C for 5 min. The reaction was then started by adding NADH and NADPH, incubation continued for a further 20 min when the reaction was stopped by extracting the reaction mixture with 5 mL of methylene chloride. The metabolism of progesterone was shown to be linear over the time of the assay. Extraction with methylene chloride was repeated and then the solvent evapourated using low heat and a stream of air. The progesterone and its metabolites were redissolved in methanol for analysis by HPLC. A Supelcosil™ LC-18 HPLC column (25 cm x 4.6 mm, Supelco Canada Ltd., Oakville, Ontario) was used with a mobile phase of 60% acetonitrile/20% H_2O delivered at 1 mL/min The radioactive metabolites were measured on line using a Beckman 171 Radioisotope Detector after mixing the mobile phase with scintillation fluid (Ecolite, ICN, California, U.S.A.) using a Beckman 110B Solvent Delivery Module (Beckman Instruments, Palo Alto, CA). The effects of interassay variance were negated by including littermates from each treatment in each assay.

2.2.10 Determination of P450 enzyme levels

A small quantity of sodium dithionite was added to 2 mL of a microsomal suspension containing 1 mg/mL of liver microsomes in 0.2 M potassium phosphate buffer (pH 7.4) and gently mixed by inversion using parafilm. The mixture was poured into a cuvette and the absorbance (A) measured at 450 and 495 nm. Carbon monoxide was bubbled through the solution for 15 seconds and the absorbance readings repeated. Concentration of P450 enzymes was calculated from,

$$\underset{\text{(nmol/mg microsomal protein)}}{\text{Cytochrome P450 content}} = \frac{\Delta A\ (450\text{-}495)\text{nm}}{1} \times \frac{1000}{91}$$

Extinction coefficient of cytochrome P450 = 91 cm^2/mmol.

$\Delta A = A_{450} - A_{495}$

The effects of interassay variance were negated by including littermates from each treatment in each assay.

2.2.11 Experimental design and statistical analyses

This experiment had a randomized block design with family as the block for the infused gilts. Within each block one gilt was assigned to each of the feed intake by body type combinations. In addition high intake infused gilts were compared with non-infused gilts using an unbalanced randomized block, again with family as the block but with body type, whether or not the gilt was infused and the interaction between these variables as the sources of variation.

Data from infused gilts, for BW, P2 backfat thickness, liver weight, lean body mass, feed intake, MCR, disappearance rate constant, half-life, P450 enzyme concentration in hepatic microsomes and ability of hepatic microsomes to metabolise progesterone, were analysed as a randomised block using analyses of variance. Sources of variation were family (=block), feed intake level (high and low), body type (lean or fat) and the interaction between feed intake and body type. The data was analysed using the general linear model procedure of Systat (Wilkinson, 1990). Comparisons between the feed intake by body type interaction means were made using least significant difference (Ott, 1993). Pearson correlations were made between fatness level and MCR, disappearance rate constant and half-life of progesterone (Ott, 1993). Comparison between preprandial and postprandial MCRs of progesterone was made as a split plot within the general linear model described above (Wilkinson, 1990). Preliminary data analyses indicated that none of the variables measured differed significantly in response to pubertal state and therefore this source of variation was not included in the final analyses.

2.3 Results

There were no interactions between feed intake and pig body type for any of the traits measured in this experiment.

By the end of the experiment F gilts were both fatter and heavier than Ln gilts (see Table 2.2, $P < 0.001$), 20.6 versus 14.0 mm P2 backfat and 137.6 versus 124.1 kg liveweight respectively.

Gilts within each rearing group had similar liveweight and fatness prior to ovariectomy, however, after two weeks of high or low level feeding liveweights within each rearing group had diversified. Gilts which received high level feeding not surprisingly grew more rapidly than those on low level feeding so that, at slaughter, these animals were significantly heavier than the low intake animals ($P < 0.001$), 135.5 versus 126.1 ± 2.21 kg respectively. There was no difference in P2 backfat between the two feed intake treatments and the mean P2 fatness was 17.3 ± 0.88 mm. As a result of the disparity in growth rates between treatment groups body protein mass differed significantly between both feed intake levels and rearing treatment ($P < 0.001$, Table 2.2). The overall mean body protein mass was 18.3 ± 0.27 kg. Daily feed intakes also differed significantly between groups but all gilts received equivalent intakes in terms of their maintenance requirements.

2.3.1 Progesterone concentration and metabolic clearance rate

Plasma progesterone levels were barely detectable in the ovariectomised gilts (see Table 2.3) prior to progesterone supplementation. After intramuscular injection of progesterone plasma levels rose rapidly and with continued progesterone supplementation had reached a steady state by the last 24 h of infusion. This is illustrated for one of the gilts in Figure 2.2 which also shows the diurnal variation in plasma progesterone levels. Plasma progesterone levels ranged between 3.0 and 18.1 ng/mL during the infusion period and were significantly different between feed intake treatments ($P < 0.01$). We were unable to establish meaningful progesterone levels in backfat due to problems in extracting progesterone from backfat and the small quantities of sample.

MCRs are expressed in terms of kg BW so that they can be readily compared with other estimates in the literature. Expression of MCRs in terms of kg body protein mass made no difference to the results. MCR of plasma progesterone was significantly increased by increasing the feed intake level and was significantly greater postprandially than preprandially ($P < 0.001$, see Table 2.4). As intake increased from 1.15 x maintenance to 2.3 x maintenance MCR increased by 23.7%, from 63.7 to 78.8 mL/min/kg BW, in preprandial animals ($P < 0.05$) and by 35.5%, from 76.1 to 103.0 mL/min/kg BW, in postprandial animals, respectively ($P < 0.01$). The effect of feeding was greater in high intake gilts with an increase in MCR of 30.7% between preprandial and post prandial measurements, versus 19.3% in the low intake gilts ($P < 0.01$).

There was no effect of rearing treatment or absolute fatness on MCR.

There was no difference in MCR between those gilts which had reached puberty prior to ovariectomy and those which had not.

2.3.2 Disappearance rate constant and progesterone half life

Rate of decline of progesterone following the end of the infusion is shown for two of the gilts in Figure 2.3. It was not possible to derive a significant regression equation for all gilts due to fluctuations in the plasma progesterone decline patterns of some animals and therefore data from 34 animals were used in this analysis. There were no significant treatment effects on the disappearance rate constant (see Table 2.4). The overall mean value for k was -0.02 ± 0.003 which is equivalent to a half life of 36.5 h, however due to the wide variability in the data the mean calculated half life was 43.1 ± 2.51 h. Half lives ranged from 8.6 to 70 h, however only four of the 34 animals had progesterone half lives which lay outside the range 31 to 60 h. There were negative correlations between liveweight and both the disappearance rate constant and half life ($P < 0.05$), however these correlations were due to three of the outlying animals and did not exist when these animals were omitted from the analysis. Interestingly the three outlying animals were all from the same family.

2.3.3 Liver weight at slaughter

Liver weight, expressed as a percentage of total body weight, was significantly increased by two weeks of high level feeding compared two weeks of low intakes feeding (see Table 2.2). Infused gilts had larger proportional liver size than non-infused gilts. Only high intake gilts were used in this comparison since all non-infused gilts were on high feed intakes. Neither absolute liver weight nor proportional liver size were correlated with MCR.

2.3.4 Liver microsomal metabolism of progesterone and P450 enzyme concentrations

When progesterone was incubated in the presence of 1 mg hepatic microsomal protein HPLC analysis revealed four main peaks. Peaks occurred at approximately 4 (peak 1), 8 (peak 2), 10 (peak 3) and 12 (peak 4) min. Peak 3 was identified as the progesterone peak, the remaining three peaks, peaks 1, 2 and 4, were progesterone metabolites which have not been identified but which are referred to here as metabolites 1, 2 and 3 respectively.

Neither feed intake level nor the rearing regime of the gilts affected the rate at which progesterone was metabolised by liver microsomes or the appearance rates of the three metabolites (see Table 2.5). The mean rate of metabolism of progesterone was 3.6 ng (0.035 nmol) /min/mg microsomal protein. There were no treatment effects on the concentration of P450 enzymes in hepatic microsomes with an overall mean concentration of 0.73 ± 0.039 nmol/mg microsomal protein (Table 2.6). Concentration of P450 enzymes was highly correlated to rate of microsomal progesterone metabolism ($P < 0.001$, $R^2 = 0.335$) but was not correlated with MCR, feed intake, liveweight, fatness or liver weight of the gilts.

Injection of pigs with progesterone for two consecutive days followed by infusion of progesterone for 60 h did not affect the concentration of P450 enzymes in hepatic microsomes isolated from livers collected 83 h after the cessation of infusion.

Nor did progesterone treatment influence the ability of P450 enzymes to metabolise progesterone when compared to that of hepatic microsomes isolated from livers of non-infused gilts (Table 2.7).

2.3.5 Effect of puberty attainment

Presumably as a result of the different boar exposure treatments and housing provided to different family groups during induction of puberty only 21 of the 40 infused gilts had reached puberty prior to ovariectomy. There was no difference in liveweight or fatness between gilts which reached puberty and those which did not. There were no differences in any aspect of progesterone metabolism between pre- and postpubertal gilts.

2.3.6 Family effects

There were significant differences between families in metabolic clearance rate ($P < 0.001$) and disappearance rate constant of progesterone ($P < 0.01$), liveweight at slaughter ($P < 0.001$), proportional liver weight ($P < 0.05$), lean body mass ($P < 0.01$) and ability of hepatic microsomes to metabolise progesterone ($P < 0.01$). Family did not affect concentration of P450 enzymes in hepatic microsomes, or pig fatness.

2.4 Discussion

MCR of progesterone is responsive to both time of feeding and feed intake; increasing the feed intake of ovariectomised gilts increased the MCR of progesterone both preprandially and postprandially. MCR increased after feeding in high and low intake gilts and this effect was greater in the high intake animals. Therefore these results confirm those of Prime and Symonds (1993) and indicate that plasma progesterone levels might be manipulated by adjusting the feed intake of sows at appropriate times. This has already been attempted in the immediate post-mating period with varying success (e.g. Ashworth, 1991; Pharazyn *et al.*, 1991; Jindal *et al.*, 1996). Increasing feed intake in sows during late pregnancy should increase progesterone metabolism to cause a more rapid decline in progesterone around farrowing with resultant successful establishment of lactation.

Plasma progesterone levels resulting from an infusion of around 130 mg progesterone per d were comparable to those reported by Prime and Symonds (1993) with similarly infused gilts. In 120 kg intact pregnant gilts, Christenson *et al.* (1985) estimated progesterone production rate to be around 112.1 mg per day, or of the same order of magnitude as the infusion rates in the current experiment. This corresponded to a mean plasma progesterone concentration of 14.4 ng/mL which again is of the same order of magnitude as the plasma progesterone levels reported in the present experiment. The slightly lower plasma progesterone levels obtained in the present study are probably because of the larger body size of the gilts (120.3 - 143.1 kg liveweight).

The lower progesterone concentrations I observed may also be because the infused progesterone was dissolved in ethanol. In this form progesterone may be more readily extracted from blood because it may remain in the ethanol as free progesterone which would be readily absorbed from plasma, whilst a lower proportion may become bound to cortisol binding globulin (CBG). CBG-bound progesterone is not available for hepatic extraction and therefore the proportion of progesterone bound to CBG determines its MCR to some extent. Normally hepatic extraction of progesterone is around 90-95%, since five to ten percent of the progesterone is bound to CBG (Bedford *et al.*, 1974; Parr *et al.*, 1993b), however, when a pharmacological bolus of 20 mg progesterone in absolute ethanol was given directly into the portal vein, the entire dose was removed in the first pass through the liver (Symonds *et al.*, 1994)

Mean progesterone MCRs obtained in the present experiment were higher than some other estimates for gilts, varying between 62.3 and 105.8 mL/min/kg BW compared to 37.8 to 67.6 mL/min/kg BW by Prime and Symonds (1993) and 44 to 51 mL/min/kg by Christenson *et al.* (1985), but they were lower than the 113 to 197 mL/min/kg reported by Henry (1985, cited by Prime and Symonds, 1993). The MCRs observed in the current study are probably higher because blood was sampled from the jugular vein and not from an artery. Steady state progesterone concentration in the jugular is always lower than that in arteries because the head region extracts between 25% (Bedford *et al.*, 1972) and 42.5% (Parr *et al.*, 1993b) of progesterone from the blood passing through it. When the MCRs obtained in this experiment are adjusted for extraction of progesterone across the head they are very similar to those obtained by Prime and Symonds who sampled from the carotid artery. For example, assuming a cross head extraction of 40%, MCR values would range from 37.4 to 63.5 mL/min/kg BW. It must be remembered that these MCR values are plasma volumes. Whole blood generally has a lower progesterone concentration than plasma as a result of the low affinity of progesterone for erythrocytes and other blood cells (Lin *et al.*, 1972; Christenson, 1984) and therefore the progesterone concentration of whole blood is lower than that of plasma (Little *et al.*, 1966), this in turn corresponds to a higher MCR. Christenson *et al.* (1985) found that MCR estimates were consistently 27% lower for plasma than for blood regardless of age or physiological state of the gilt. If the MCRs obtained in the present study are corrected for haematocrit in addition to the correction made for clearance across the head they approach the original values given in the results.

Neither rearing treatment nor backfat thickness *per se* affected MCR. In addition to storing progesterone, in humans at least, adipose tissue is also able to metabolise a proportion of it (Bleau *et al.*, 1974). However, adipose tissue appears to metabolise progesterone at a similar rate to the other nonsplanchnic tissues because level of fatness had no overall effect on MCR in the present experiment.

Despite efforts to have all gilts cyclic prior to ovariectomy this was achieved in only 21 of the 40, however, there was no difference in MCR of progesterone regardless of whether gilts had attained puberty or not. This is in agreement with Christenson *et* *al.* (1985), who showed that MCR was greater in lighter, immature gilts but above 95 kg there was no difference in MCR of progesterone regardless of whether gilts were prepubertal, pubertal or pregnant.

The response of liver blood flow rate to feeding is dramatic with increases of up to 172% above the fasted level (fasted for 48h) within 2 h of feeding (White *et al.*, 1973). Although the response to varying feeding levels is likely to be less extreme it may be sufficient to account for corresponding variations in MCR. However, if MCR increases by 30 to 35% then, assuming non-splanchnic clearance equals 50% of splanchnic clearance, liver blood flow must increase by 60 to 70% to account for the increased clearance. The only portal blood flow data at different feed intake levels currently available for pigs are those of Prime and Symonds (1993), which unfortunately lack credibility because they are unrealistically low values. If these values, although low, are proportionately correct they show a similar relative increase to that of MCR in response to high versus low intake feeding, both increasing by around 45%. As indicated previously this increase alone would be insufficient to account for the difference in MCR observed. However the uncertainty of the blood flow data make this conclusion premature. Certainly in sheep, the increase in liver blood flow in response to increased feeding level is not in itself sufficient to account for the whole of the corresponding increase in MCR of progesterone (Parr *et al.*, 1993a, b).

Liver size was significantly increased by increasing feed intake and also by the infusion itself, perhaps in response to ethanol. However the ability of the liver, per unit microsomal protein, to metabolise progesterone was unchanged by either increasing the fatness of the animal or increasing its feed intake, nor was there an increase in the concentration of cytochrome P450 enzymes in response to the various treatments. Any increase in hepatic ability to metabolise progesterone would therefore be due to the absolute increase in liver size and not to up-regulation of enzyme systems. The livers of high intake gilts were 13% larger than those of low intake animals and hence had 13% greater capacity to metabolise progesterone. It is likely that if liver blood flow is increased then greater liver capacity will be required, although not necessarily for progesterone metabolism. The liver already has a tremendous capacity to metabolise progesterone and it has been demonstrated that injection of supraphysiological levels of progesterone into blood entering the liver has no effect on the low levels of progesterone in the hepatic vein (Symonds *et al.*, 1994). It would be interesting to know whether MFO systems in other tissues are up-regulated by increasing feed intake, since hepatic blood flow regulation may not be the only cause of increased progesterone MCR in high intake animals. Parr *et al.* (1993b) found that percentage extraction rates by the head and the mesenteric/intestinal regions remained similar regardless of feed intake in sheep, suggesting that MFO capacity remains unchanged in response to feeding in these tissues.

Ovariectomising gilts and then infusing them with progesterone did not cause up-regulation of progesterone metabolising systems. When hepatic microsomes from infused gilts were compared to those from non-infused gilts there was no difference in P450 enzyme concentrations nor in their ability to metabolise progesterone. Admittedly the liver samples were taken at slaughter approximately 83 h after termination of the progesterone infusion but all except two of the gilts still had measurable quantities of progesterone in blood 12 h before slaughter. Likewise there were no differences in MFO concentration and activity between pubertal and prepubertal gilts. It therefore seems that levels of hepatic P450 cytochrome enzymes and their ability to metabolise progesterone are remarkably stable in mature gilts, since they were unaffected by any of the factors investigated in this experiment.

One surprising finding in this work was that the high MCR during progesterone infusion was followed by a long half life of 31 to 60 h after the infusion ended. There are few estimates of progesterone half life in pigs in the literature. Hillbrand and Elsaesser (1983) measured the decline in progesterone immediately following ovariectomy and found that plasma progesterone initially fell rapidly but then more gradually. Plasma progesterone levels increased during surgery immediately prior to ovariectomy as a result of a surge in progesterone in response to the anaesthetic. After ovariectomy plasma progesterone fell from its initial level of 34.2 ng/mL to half that value within 120 min but the next half life was twice as long and subsequent half lives became increasingly longer until the last one, measured between d 3 and d 6 after the ovariectomy, which was 49 h. Half life length in backfat was longer than that in plasma, initial half life was estimated to be 34 h but, as with plasma, half lives grew longer as progesterone concentration declined. Half life is a measure of the rate of disappearance of progesterone from all pools in the body plus the interactions between them. It is therefore under multifactorial control.

In the present experiment the length of half life was not affected by feed intake or level of fatness. This agrees with the work of Hillbrand and Elsaesser (1983) who also found no effect of fat to lean ratios on progesterone levels or rate of change following ovariectomy. However it is surprising that when feed intake had significant effects on MCR it should have no effect on half life. This is partly due to the large variation between observations since there was an overall trend for high intake gilts to have shorter half lives than low intake gilts, 44 versus 51.9 h, respectively.

With the exception of two gilts with half lives of around 8 h, half lives were longer than expected in this experiment although not dissimilar to those observed by Hillbrand and Elsaesser (1983) at lower plasma concentrations. The reduced effect of feed intake on progesterone metabolism following withdrawal of progesterone infusion suggests that a greater proportion of the remaining progesterone is unavailable for metabolism, perhaps due to increased binding to CBG. It is possible that ovariectomy caused a change in the balance of plasma proteins. However, it seems unlikely that it could have induced an increase in CBG levels since the main promoter of CBG levels is oestrogen (Rosner, 1991), unless of course progesterone itself induces increased CBG levels in pigs. An increase in CBG concentration is not the only thing which would cause increased progesterone binding to CBG. Binding would also increase if progesterone affinity for albumin were reduced. Cortisol and progesterone have the same affinity for CBG but progesterone has higher affinity for plasma albumins (Siiteri *et al.*, 1982), as a result a greater proportion of progesterone than of cortisol binds to albumins. Since albumin-bound steroid is readily extracted from the blood the MCR for progesterone is ten times greater than that for cortisol (Siiteri *et al.*, 1982). Changing plasma protein levels following ovariectomy would also explain the gradual increase in half-life observed by Hillbrand and Elsaesser (1983).

The results of the present experiment, in combination with those of Hillbrand and Elsaesser (1983), suggest that once plasma progesterone levels have become fairly low they decline slowly, presumably maintained by progesterone coming from adipose tissue. There may also be proportionately greater binding of progesterone to CBG making progesterone less available for metabolism, and hence increasing progesterone half life. At this stage there may still be quite high progesterone levels in adipose tissue. Six days after ovariectomy backfat contained 63 ng progesterone/g fat whilst plasma levels had fallen to 0.5 ng/mL (Hillbrand and Elsaesser, 1983). The slow decline of progesterone concentration in backfat is itself surprising. A gilt with around 30 ng progesterone/mL plasma will have a concentration of 90 ng progesterone/100 mg backfat, corresponding to 36 mg progesterone stored in adipose tissue. Although this is equivalent to the maximum daily production of the corpora lutea, as measured by

Brinkley and Young (1968), it is much lower than the daily progesterone production rate of 155.3 mg/d (day 12 of the oestrous cycle) calculated by Christenson *et al.* (1985). Either way it seems extraordinary that gilts which can easily metabolise 130 mg infused progesterone per d should take so long to rid themselves of a mere 36 mg. It seems possible that either adipose tissue in pigs is able to convert progesterone metabolites back into progesterone or that the progesterone concentration in adipose tissue has been underestimated.

There is an alternative explanation to the apparent anomaly of high MCR and yet long half life. In addition to Christenson *et al.*'s (1985) estimates of progesterone production rates in gilts, there are two other estimates of progesterone production rate available in the literature. Christenson *et al.* measured production rates of 155.3 ± 7.8 mg/d in 112 kg gilts on d 12 of the oestrous cycle and 112.1±13.2 mg/d for 120 kg gilts on d 32 of gestation using continuous infusion of tritiated progesterone for 175 min, with sampling for the last 30 min. Brinkley and Young (1968) estimated a total ovarian output of progesterone on d 11 of the oestrous cycle to be 38.6 mg/d by collecting the total venous output from the ovaries in 9 gilts ranging in liveweight from 105 to 138 kg. They do admit that this estimate may be low as they did not take account of any progesterone secreted into the lymph and their assay for progesterone was also quite insensitive. Stone *et al.* (1986) used continuous infusion of deuterium-labeled progesterone for 3.5h into pregnant or non-pregnant gilts, taking samples between 1.5 and 2.5 h after the start of the infusion. They determined progesterone production rates of 86.2 ± 8.2 mg/d in pregnant gilts (d11 to 17 post coitus) and 49.7 ± 3.8 mg/d in non-pregnant gilts (days 11 to 17 post oestrus). However they failed to present evidence that steady state conditions existed at the time of sampling. If the lower daily production rates of Brinkley and Young (1968), and Stone *et al.* (1986) are correct then clearly the intra-muscular priming doses given in this experiment were too high, representing around three times the maximum endogenous production rate. In this case it is possible that these injection sites were still providing a source of progesterone after the end of the infusion. This would also mean that the presumed progesterone stores in adipose tissue, calculated from Hillbrand and Elsaesser's data, would represent one day's production and therefore might be expected to maintain plasma progesterone at a reasonable level for a few days after withdrawal of the source of progesterone. There are several problems with this theory. For example, in this experiment and that of Prime and Symonds (1993), what happened to all the infused progesterone? It is possible that it is all being cleared in a single pass through the circulation. Symonds *et al.* (1994) injected a bolus of 15 mg progesterone suspended in 1 mL absolute ethanol into the anterior vena cava or the jugular vein of 73 to 80 kg gilts and, after 2 min, found less than one tenth of the expected plasma levels. This suggests that infused progesterone was rapidly extracted from the blood as indeed the results of my own experiment have proved. The validity of this alternative hypothesis is questionable because, although there was high first pass clearance of progesterone in Symonds *et al.*'s (1994) gilts, some progesterone did remain in the blood and this would gradually have increased over time with continued infusion. In the current trial the two preliminary gilts initially received no priming injections, but nevertheless showed a slow but steady increase in plasma progesterone in response to 5 d of continuous infusion of 130 mg progesterone/d. Most significantly, the two preliminary gilts had similar progesterone disappearance rates to the rest of the gilts in this trial after the infusion of progesterone had stopped. Obviously this could not have resulted from continued progesterone release from intra-muscular injection. If three times normal progesterone production rates were being supplied then it is unlikely that plasma progesterone levels would remain so low. As discussed earlier, plasma levels in this experiment were, if anything, lower than expected and certainly do not support the idea of supraphysiological doses of progesterone being supplied.

Enterohepatic circulation provides another possible explanation for the slow decline in plasma progesterone levels. This is the recirculation of progesterone back into the body after being excreted into the intestine via the bile (Aldercreutz and Martin, 1980). Enterohepatic circulation has been demonstrated in the pig (Symonds *et al.*, 1994) but it is unlikely to contribute much progesterone to the general circulation. Reabsorbed progesterone enters the mesenteric vein and hence the portal vein so that 90% of it is immediately cleared again by the liver.

Plasma progesterone levels might also remain high following the cessation of progesterone infusion if the adrenals were producing significant amounts of progesterone. However, this is improbable since pre-infusion concentrations of progesterone were barely detectable indicating that adrenal production was extremely low. It is unlikely that adrenal production has suddenly dramatically increased in response to progesterone infusion and therefore I assume that it has remained at the pre-infusion production level.

In this experiment there were a number of apparent differences in progesterone metabolism between families. These included significantly different metabolic clearance rates, disappearance rate constants and hepatic microsomal ability to metabolise progesterone. Similar variance in hormonal and reproductive traits between families have been reported by Cosgrove *et al.* (1991) and Beltranena *et al.* (1993), who noticed significant effects of litter of origin on LH secretion and attainment of puberty in gilts, respectively. It is exciting to postulate that such genetic differences in hormonal metabolism may be responsible for the inherent differences in reproductive performance between individual sows. However, in the present experiment we must be careful not to place too great an inference on these findings since all laboratory analyses were performed using one or more family group per replicate. Therefore, any differences between replicates were included with any inherent differences between litters and may have exaggerated inherent genetic differences. Nevertheless this data suggests that there are significant genetic differences in progesterone metabolism.

In conclusion, MCR of progesterone was shown to be increased by increasing the feed intake of ovariectomised gilts so that adjustment of feed intake could be used as a management tool to manipulate plasma progesterone concentrations in sows. This effect is assumed to be mediated largely by an increase in hepatic blood flow. The effects of feed intake on the rate of decline of progesterone following removal of the progesterone source were less obvious. This was thought to be due to a change in the proportion of progesterone binding to CBG and hence becoming less available for extraction as a result of the ovariectomy. P2 backfat thickness and the way in which gilts were reared did not affect any aspect of progesterone metabolism measured in this study. MFO levels in hepatic microsomes and their ability to metabolise progesterone were stable regardless of feeding level or physiological state of the gilt. There were significant litter differences in progesterone metabolism suggesting that appropriate selective breeding could influence this variable in sow herds.

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Table 2.1. Diets used to grow gilts from 30 kg BW to target weight. Grower diets were fed from 30 kg to 50 kg BW and finisher diets for the remainder of the period.

Ingredients (%)	Grower High energy	Grower Low energy	Finisher High energy	Finisher Low energy
Barley	35.44	40.27	32.88	38.30
Wheat	40.00	31.22	45.00	37.86
Soybean meal	12.57	23.84	10.43	19.05
Tallow	6.09	0	6.06	0
Canola oil	1.80	0	1.80	0
Dicalcium phosphate	1.20	1.60	1.20	1.60
Limestone	1.10	1.30	1.10	1.30
Salt	0.40	0.50	0.40	0.50
Mineral/vitamin premix[1]	1.00	1.025	1.00	1.25
HCL-lysine	0.307	0.205	0.130	0.095
DL-methionine	0.009	0.021	0	0.045
Threonine	0.084	0.019	0	0
Nutrient composition				
% Dry Matter	88.99	88.16	88.89	88.03
Digestible Energy (MJ/kg)	14.89	13.19	14.89	13.19
% Crude Fibre	3.73	4.56	3.58	4.29
% Crude Protein	16.00	20.20	14.50	18.10
% Lysine	0.92	1.14	0.72	0.93

[1] Supplied the following per kg of diet: Zinc, 120mg; Manganese, 12 mg; Iron, 150 mg; Copper, 12 mg; Selenium, 0.1 mg; Vitamin A, 5000IU; Vitamin D_3, 500 IU; Vitamin E, 22 IU; Riboflavin, 12 mg; Niacin, 45 mg; Calcium Pantothenate, 24 mg; Choline Chloride, 840 mg; Vitamin B_{12}, 30 µg; Biotin, 200 µg.

Table 2.2 Liveweight, fatness and liver weight of gilts reared to be either fat or lean and fed either 2.3 or 1.15 x maintenance, at slaughter.

	HF	HLn	LF	LLn	SEM
Liveweight (kg)	143.1^{a}	127.9^{b}	132.0^{c}	120.3^{d}	2.79***
P2 backfat (mm)	20.6^{a}	14.5^{b}	20.6^{a}	13.6^{b}	1.46***
Liver wt (g/kg BW)	13.5^{a}	13.6^{a}	12.3^{b}	11.7^{b}	0.34***
Body protein mass (kg)	19.9^{a}	18.4^{b}	17.8^{c}	17.1^{d}	0.45**
Daily feed intake (kg)	3.1^{a}	2.8^{b}	1.6^{c}	1.5^{d}	0.033***

Figures with different superscripts within the same row are significantly different, * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

Table 2.3 Plasma progesterone concentration (ng/mL) of gilts reared to be either fat or lean and fed either 2.3 or 1.15 x maintenance.

Plasma progesterone	HF	HLn	LF	LLn	SEM
pre supplementation	0.07	0.05	0.09	0.13	0.027
During infusion					
Preprandial	8.11^{a}	8.45^{a}	10.20^{b}	11.20^{b}	1.081
Postprandial	6.58^{a}	6.46^{a}	7.97^{b}	9.38^{b}	0.913

Figures with different superscripts within the same row are significantly different, $P < 0.01$

Table 2.4 Metabolic clearance rate of progesterone, disappearance rate constant and progesterone half life in gilts reared to be either fat or lean and fed either 2.3 or 1.15 x maintenance

	HF	HLn	LF	LLn	SEM
Preprandial MCR	77.1^{a}	80.5^{a}	65.1^{b}	62.3^{b}	9.23*
Postprandial MCR	100.2^{a}	105.8^{a}	78.56^{b}	73.5^{b}	1.76**
Disappearance rate constant	-0.018	-0.022	-0.023	-0.014	0.006
Half-life (h)	43.8	44.2	50.7	53.1	7.43

MCRs expressed in mL/min/kg BW.
Figures with different superscripts within the same row are significantly different, * $P < 0.05$, ** $P < 0.01$

Table 2.5 Proportions of progesterone and three of its metabolites after 20 min incubation with hepatic microsomes isolated from gilts reared to be either fat or lean and fed either 2.3 or 1.15 x maintenance.

	HF	HLn	LF	LLn	SEM
Progesterone	67.5	64.4	63.7	66.0	1.98
Metabolite 1	7.8	8.3	7.5	8.3	0.60
Metabolite 2	13.1	12.8	12.2	12.0	0.86
Metabolite 3	9.9	14.6	13.5	12.5	1.78

Table 2.6 P450 enzyme concentrations (nmol/mg microsomal protein) in hepatic microsomes isolated from gilts reared to be either fat or lean and fed either 2.3 or 1.15 x maintenance

	HF	HLn	LF	LLn	SEM
P450 enzymes	0.692	0.735	0.722	0.774	0.064

Table 2.7 Effect of progesterone (progesterone) infusion on the P450 enzyme concentration of hepatic microsomes and their ability to metabolise progesterone

	Progesterone peak	M1 peak	M2 peak	M3 peak	P450 enzyme
Infused	64.7	8.0	12.6	12.9	0.735
SEM	1.07	0.33	0.48	1.01	0.03
Non-infused	68.8	7.5	12.3	10.8	0.754
SEM	2.14	0.65	0.95	2.02	0.06

Figure 2.1 Blood sampling times following the end of the progesterone infusion

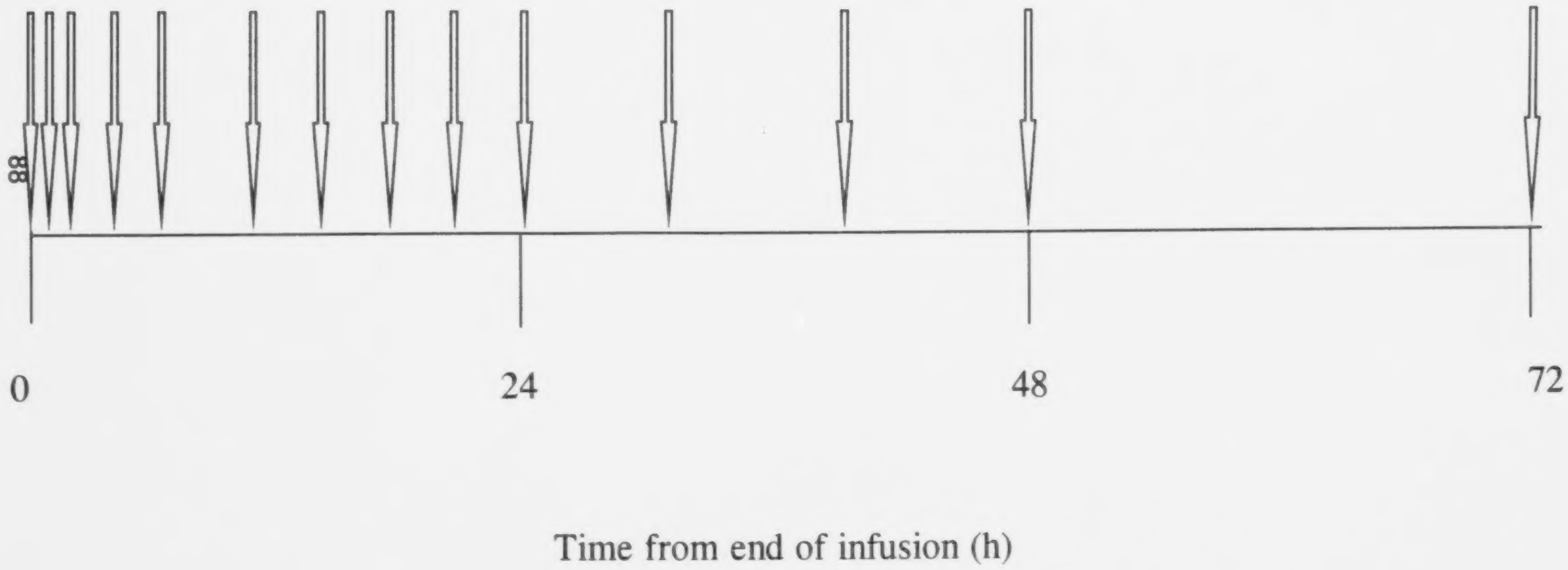

0
24
48
72
Time from end of infusion (h)

Figure 2.2 Diurnal pattern of plasma progesterone in one progesterone infused gilt

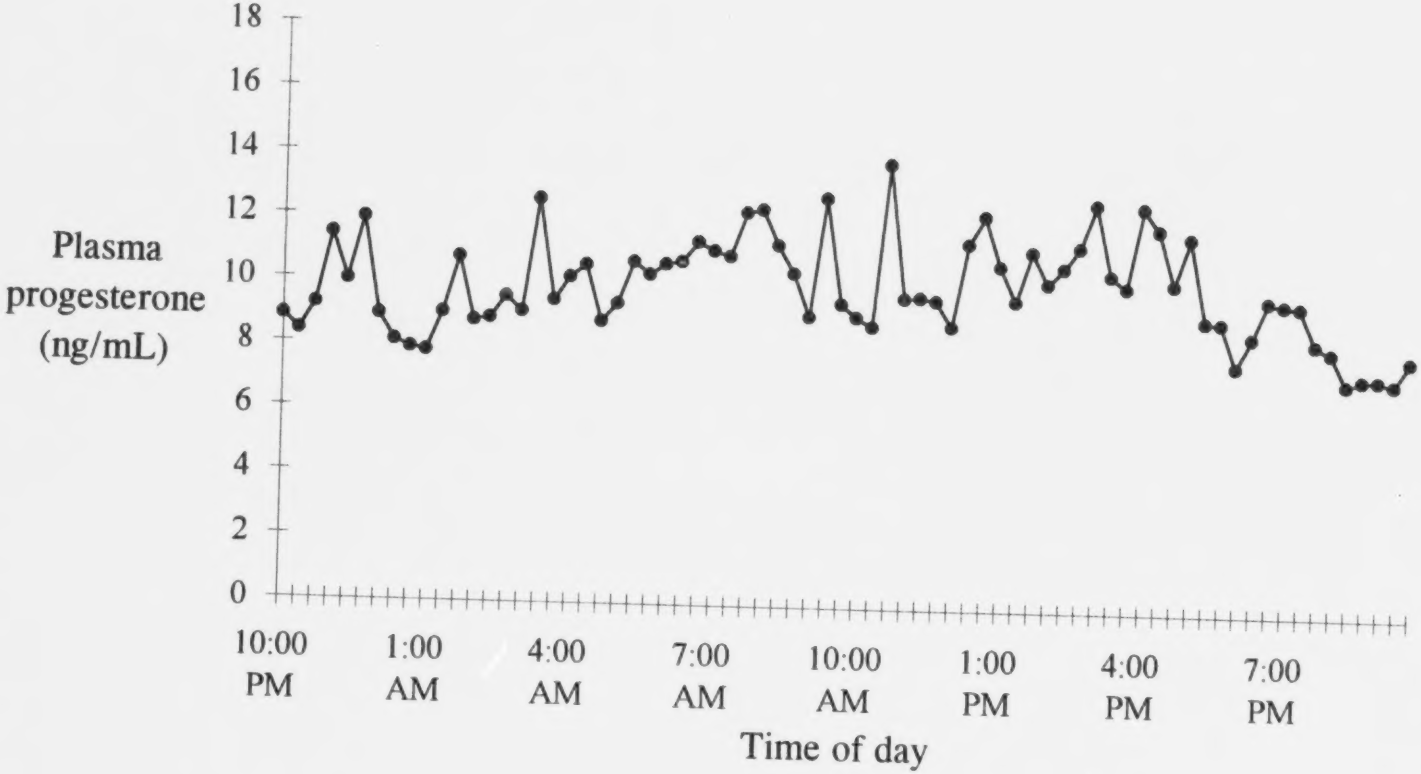
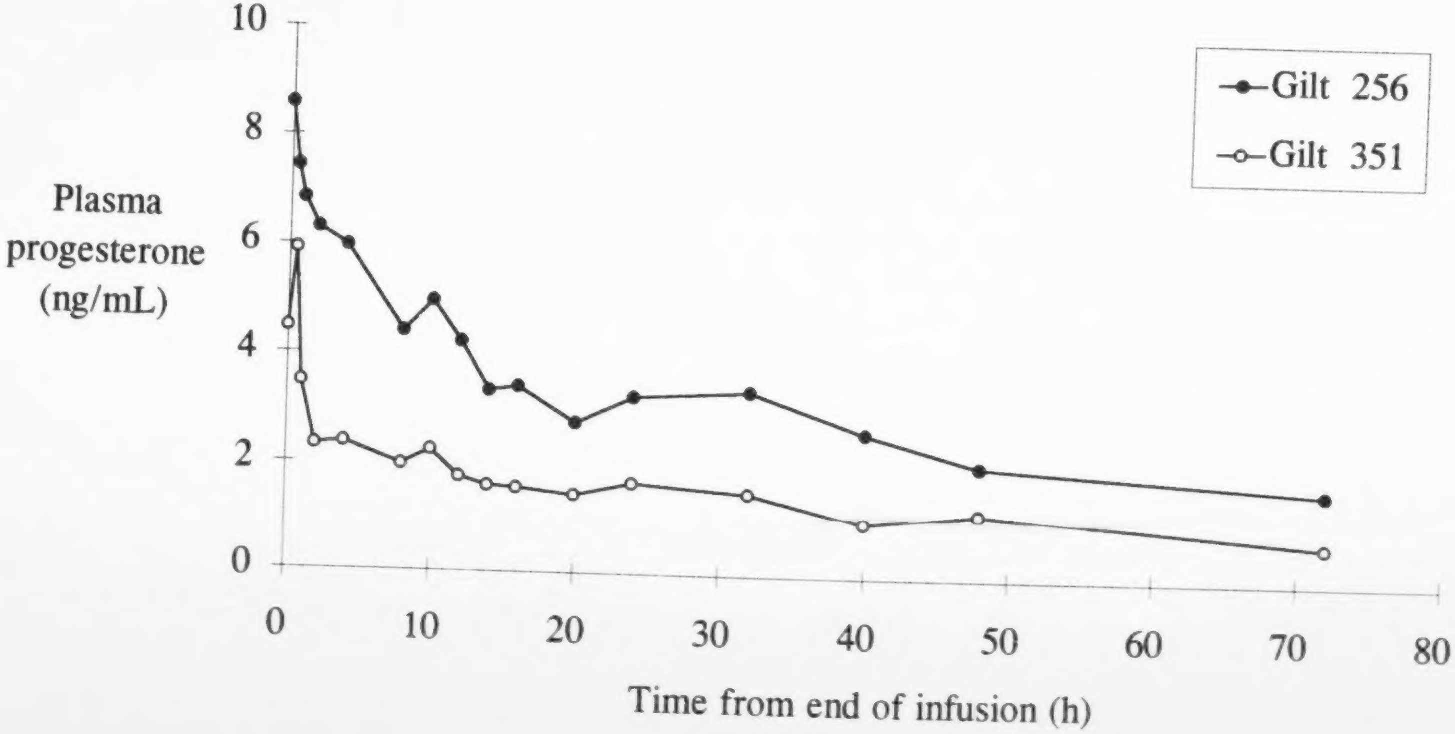


Figure 2.3 Rate of decline of plasma progesterone following termination of infusion



CHAPTER 3

The Effects of Varying Feed Intake in Late Gestation on Sow Performance in Early Lactation and on Piglet Viability and Growth Rate

3.1 Introduction

3.1.1 Possible implications of increasing feed intake in late gestation

3.1.1.1 Gestation

Conventional gestation feeding provides a feed allowance to meet the nutrient requirement of the sow for maintenance and a targeted weight gain appropriate for that parity. This target weight gain consists of a desired weight increase for the sow plus an allowance for the products of conception. The total nutrient requirements for maintenance and target weight gain are averaged over gestation and the sow is fed a constant daily allowance throughout. However, the nutrient requirements of sows increase as pregnancy progresses in relation to the pattern of fetal development and increased BW of the sow. Fetal growth rate increases exponentially throughout gestation rising from 66 g/d on d 50 to 290 g/d by d 110 (Noblet *et al.*, 1985). Therefore nutrient requirements of the sow are greatest in late gestation when fetal growth is greatest and the sow is approaching her maximum liveweight. Sows receiving a gestation allowance of less than at least 25 MJ DE/d may lose body reserves, primarily fat, in the last 2 to 3 weeks of pregnancy (Close *et al.*, 1985; Cole, 1990;

Noblet *et al.*, 1990) unless their feed intake is increased during this period. Close *et al.* (1985) found that sows receiving 20 MJ ME/d were in negative energy balance by d 87 of gestation and were estimated to be losing 140 g fat per d by term. Whittemore (1993) stresses the importance of maintaining at least 12 mm P2 backfat on the sow at weaning or sow reproductive performance will be compromised. Most sows lose backfat during lactation (Mullan, 1991; Aherne *et al.*, 1991) and therefore with modern lean genotypes it may be crucial to maintain sow fatness in late gestation in order to maximise sow output and longevity in the breeding herd.

Protein requirements also increase with advancing pregnancy. Noblet *et al.* (1985) estimated that the nitrogen retention in the conceptus and udder increased from 2 g/d in mid-pregnancy to 14 g/d in late gestation. Everts (1994) has shown that, whilst an intake of 42 g N (262.5 g protein) per d in mid gestation was sufficient to maximise nitrogen retention in the products of conception and in the sow (10 g N/d), 50 g nitrogen (312.5 g protein) per d in late gestation was enough to meet the nitrogen retention demands of the products of conception but limited maternal nitrogen retention to 4 g N/d, compared to 13.5 g N/d in sows fed 77 g nitrogen (481 g protein) per d. It is therefore not surprising that sows which received increased feed intake in late gestation gained more weight in the last few weeks before farrowing than flat rate fed controls (Sterling and Cline, 1986; Cromwell *et al.*, 1989).

3.1.1.2 Piglet birthweight

Seventy percent of piglet mortalities occur in the first three days of life, often as a result of low birthweight (Dyck and Swierstra, 1987). However, efforts to increase birthweight of piglets by increasing sow feed intake in late gestation have been unimpressive and variable (Aherne and Kirkwood, 1985). Hillyer and Phillips (1980) fed multiparous sows an additional 1 kg/d from d 90 of gestation until farrowing but found no significant effect on any aspect of subsequent litter performance when compared to sows fed a flat rate of 2.4 kg/d throughout. Sows which were fed *ad libitum* from d 105 of gestation until farrowing produced piglets with similar birthweights to those of sows fed 1.8 kg/d throughout, but higher intake sows had a greater number of stillbirths (Stirling and Cline, 1986). Cromwell *et al.* (1989) did see a response to increasing feeding level of sows in late gestation. High intake sows produced larger litters of heavier birthweight and subsequently higher weaning weight, however these effects were marginal and only became significant for sows which had received high intake feeding for at least two consecutive parities. Therefore it seems that the major advantage of increasing sow feed intake in late gestation is in maintaining sow condition prior to lactation rather than in conferring any birthweight advantage to her piglets.

3.1.1.3 Lactogenesis and piglet performance

Increasing feed intake of sows in late gestation may increase piglet survival and growth rate in early lactation by influencing the time of onset of lactogenesis. Piglet mortalities occurring within the first three days of life are characterised by low energy intakes resulting in low growth rates (Dyck and Swierstra, 1987). De Passillé *et al.* (1989) observed that multiple deaths within litters were correlated with poor weight gains of surviving piglets. They suggested that some sows may have difficulty establishing lactation (lactogenesis). Progesterone withdrawal is thought to be the trigger for the onset of lactogenesis in the sow (Hartmann and Holmes, 1989). A number of workers have measured plasma progesterone around farrowing (Molokwu and Wagner, 1973; de Passillé *et al.*, 1993). The general pattern observed is that after being maintained at high levels throughout gestation, progesterone declines rapidly in the 24 to 48 h before farrowing. Whereas most workers have reported mean values for all sows studied, de Passillé *et al.* (1993) looked at the variation between sows and individual sow patterns of decline in plasma progesterone. They observed considerable variation between individuals and found that there was a negative correlation between sow plasma progesterone levels during the first 48 h after farrowing and piglet growth rates in the first three days of life. This suggests that the rate of decline of progesterone after farrowing may be crucial to the successful establishment of early lactation.

We have already established that increasing feed intake level increases metabolic clearance rate (MCR) of progesterone in ovariectomised gilts (see Chapter 2). Therefore, increasing the feeding level of sows in late gestation should increase MCR of progesterone, thus reducing prefarrowing plasma levels and hastening the rate of decline of progesterone at farrowing. This in turn should lead to earlier onset of lactogenesis. As already noted, although circulating levels of progesterone are variable around farrowing the reasons for this are largely unknown, although there is a positive correlation with litter size (de Passillé *et al.*, 1993). The corpora lutea are the main source of progesterone in the pregnant sow (Craig, 1982) and these regress before parturition (Edwards, 1982) resulting in low or zero production of progesterone at this time. Craig (1982) has shown that a small amount of progesterone is produced by the feto-placental unit but this is insufficient to support pregnancy in the absence of the corpora lutea and the placenta becomes detached at the end of farrowing. Progesterone is stored at high concentrations in adipose tissue (Hillbrand and Elsaesser, 1983) so that sow fatness may also affect the rate of decline of progesterone at farrowing, although this was not substantiated in earlier work with ovariectomised gilts (see Chapter 2) nor in the work of Hillbrand and Elsaesser (1983). In both of these studies the level of pig fatness had no effect on the rate of change of progesterone in plasma.

3.1.1.4 Lactation feed intake

It is widely known that increasing feed intake in gestation reduces feed intake in lactation (Salmon-Legagneur and Rerat, 1962, Mullan and Williams, 1989). This was most recently demonstrated by Weldon *et al.* (1994) who fed gilts *ad libitum* (3.7 kg/d) or 1.85 kg/d of a 14% protein and 0.7% lysine diet from d 60 of gestation. All sows were fed *ad libitum* throughout the subsequent 28 d lactation. There was a significant reduction in lactation feed intake for sows that had received high levels of feed in gestation. Overall feed intakes and weight changes in gestation and lactation were almost identical in both groups of sows, as was lactation performance. However, it seems unlikely that a short period of increased feeding at a time when sows would otherwise be catabolic will have a detrimental effect on lactation feed intake. Rather it might be expected that higher feeding levels in late gestation could better prepare the digestive system for high intakes during lactation. It was noted in the experiment described in Chapter 2 that 16 d of high level feeding significantly increased the proportional liver weights of ovariectomised gilts above those of gilts fed half as much. It is certain that enlargement of the alimentary tract and associated organs would also occur in sows which received higher levels of intake in late gestation. This in turn should provide them with increased digestive capacity during lactation, particularly early lactation. Sows fed high intake levels in gestation should therefore have higher diet digestibility in early lactation and should be able to increase their feed intake during lactation more rapidly. This carryover effect has been observed in gilts which were restricted (20% below NRC requirements) during growth. They exhibited reduced feed intake and lower dry matter digestibility during gestation than gilts which had been fed recommended intakes during growth (Zivkovic and Bowland, 1963). Matte *et al.* (1994) and Farmer *et al.* (1996) have shown that feeding a greater volume to sows during gestation, by using high fibre ingredients, resulted in increased lactation feed intake compared to controls. Presumably this was due to enlarged capacity of the digestive tract because both groups of sows had received similar nutrient intakes. Therefore increasing feed intake in late gestation might also cause up-regulation of the sow digestive system and lead to greater lactation feed intakes and, or improved diet digestibility.

3.1.1.5 Subsequent reproductive performance

It is widely recognised that the voluntary feed intake of sows during lactation frequently fails to meet the nutrient requirements for maintenance and milk production so that sows catabolise their own tissues to maintain an adequate lactation (Williams and Mullan, 1989; Aherne *et al.*, 1991). This can result in an extended weaning to mating interval (WEI, King and Williams, 1984) and in reduced embryo survival and subsequent litter size, particularly in first parity sows (Hughes, 1989). Work from the University of Minnesota has led to the hypothesis that nutritional deprivation in early lactation can affect the fertility of the sow after weaning (Tokach *et al.*, 1992, Koketsu, 1994). If sows enter lactation in a catabolic state as the result of feeding a constant daily allowance throughout gestation this may exacerbate the detrimental effects of low feed intakes in lactation. It is also possible that follicles ovulated after weaning may first emerge from the primordial pool of follicles during late gestation so that the metabolic state of the sow during this period may affect the integrity of the emerging follicles (Foxcroft *et al.*, 1995).

3.1.1.6 Agalactia

While increasing feed intake in late gestation appears to bring a number of potential advantages to the sow there may also be detrimental effects. Göransson (1989) fed sows either 3.4 or 1 kg/d of a concentrate ration for the last 14 d of gestation, with free access to straw. He observed a greater incidence of agalactia in the higher intake sows, 32% versus 16% in the low intake sows. Persson *et al.* (1989) performed an apparently identical experiment at the same research station over the same time period with an almost identical group of sows and obtained similar results! The incidence of agalactia in their experiment was 27% in the high intake sows versus 14% for the low intake animals. In both of these experiments the incidence of agalactia is high even in the low intake groups since the normal incidence of agalactia lies between 4 and 10 percent, however, within individual herds, the incidence can vary from zero to 100% (Aherne, 1973). A major factor determining herd incidence is the criteria used to assess the presence and severity of the syndrome. Miquet *et al.* (cited by Martineau *et al.*, 1992) suggested an index based on rectal temperature, poor appetite, mammary changes, vulval discharge and delayed parturition. In the work of Göransson (1989) and Persson *et al.* (1989) a rectal temperature greater than 39.5°C within the first 48 h after farrowing was the sole criterion used in determining whether or not sows were agalactic. Subsequent milk and mammary investigations were performed on affected sows but not on the majority of other sows in the experiments.

If agalactia is induced by high feeding levels in late gestation this may occur via increasing the MCR, and hence rate of decline, of progesterone. This in turn may cause premature initiation of lactogenesis with subsequent engorgement and inflammation of the mammary gland. Gooneratne *et al.* (1982) observed that lactose concentrations in colostrum were higher in sows that subsequently became agalactic than in those which did not. They suggested that this may indicate premature lactogenesis. One of the aims of this experiment was to determine whether increasing feed intake in late gestation does hasten the decline of progesterone at farrowing and whether this affects the time of onset of lactogenesis as determined by litter weight gain in the first two days of life.

3.1.2 Hypotheses

Based on the literature described it was hypothesised that increasing the feeding level of sows in late gestation would;

- prevent sow fat loss in late gestation.
- increase feed intake and feed digestibility in early lactation.
- result in lower plasma progesterone concentrations in late gestation and more rapid decline of these concentrations around farrowing. This in turn will enhance the onset of lactogenesis with resultant improvement in piglet performance.
- have no effect on piglet birthweight.
- improve subsequent reproductive performance.

3.2 Materials and Methods

Seventy eight Camborough sows (Pig Improvement (Canada) Ltd., Acme, AB, Canada), parities 1 to 3, were fed 1.15 x maintenance energy levels until d 100 of gestation (Normal). From d 100 of gestation until farrowing the sows were randomly allocated within parity either;

- to remain on normal feed intake level (1.15 x maintenance energy - **N sows**), or
- to receive a high feed intake (twice maintenance energy - **H sows**).

Maintenance requirement was assumed to be 460 kJ DE/kg $BW^{0.75}$ (NRC, 1988). Sows that remained in the trial for more than one parity were assigned to alternate treatments in successive parities, giving a total number of one hundred and seventeen sow records. This, combined with the limited availability of sows led to unequal numbers of sows on each treatment. The allocation of sows to treatment is shown in Table 3.1. The daily ration was fed once daily at 8 am. The gestation diet contained 12.6 MJ DE/kg and 13.7% crude protein (Table 3.2). During gestation the sows were housed in individual stalls at a room temperature of 20°C ± 2°C. On d 109 of gestation the sows were moved into individual farrowing crates in the farrowing room and from this time until the end of lactation they received an appropriate amount of the lactation diet (see Table 3.2), which contained 13.7 MJ DE/kg and 15.4% crude protein. Farrowing stalls were totally slatted and had covered creep areas containing heat lamps and overlays. Room temperature was maintained at 18°C ± 2°C. After farrowing all sows were fed *ad libitum* throughout a 25 (±0.3) d lactation. Feed remaining in the sow's trough was weighed back once daily at 7 am. Sows were then presented with new feed and additional feed was supplied at noon and 4 pm if required. Sows had access to water at all times.

All sows were weighed on d 98 of gestation, within 24 h of farrowing and at weaning. Backfat was measured on each of these days using an ultrasonic probe (Scanoprobe 11, Scanco, Ithaca, NY) at the last rib and 65 mm from the midline (P2).

Litter size was standardised across treatments within 24 h of parturition. On the d of birth, piglets received an iron supplement, their teeth and tails were clipped and their ears were notched. Male piglets were castrated at 7 d of age. Piglets were weighed within 16 h of birth and then at 1, 2, 6 and 20 d of age and at weaning. Piglets had access to creep feed from 20 d of age and to water at all times. Intake of creep feed was not recorded.

3.2.1 Blood sampling

Blood samples were taken from forty four sows, 19 H sows (5 x parity 1, 10 x parity 2 and 4 x parity 3) and 25 N sows (11 x parity 1, 8 x parity 2 and 6 x parity 3) by ear vein puncture and analysed for progesterone to determine how feeding level in late gestation affected plasma progesterone levels in late gestation and early lactation. Blood samples were taken on d 98, 2 h after feeding, d 110, immediately before and 2 h after feeding, during farrowing, 6 h later and at 9 am on the next three days (see Figure 3.1).

Every attempt was made to take blood samples without unduly stressing the sow although, prior to farrowing, it was sometimes necessary to restrain the sow with a nose snare during blood sampling. After farrowing sows remained within their farrowing crates during blood sampling and were not restrained in any other way. An elastic band was placed around the base of the ear. The ear was swabbed thoroughly with 50% ethanol and a needle inserted gently into one of the more prominent veins. 5 mL blood were extracted and transferred to a heparinised tube. The elastic band was removed from the ear and then the needle was withdrawn. Pressure was applied to the puncture site with a sterile swab for approximately one minute to prevent any further bleeding. All blood samples were centrifuged at 1500 x *g* for 15 min at 4°C within 20 min of sampling and were stored at -30 °C until analysis.

3.2.2 Progesterone analysis

Plasma samples were analysed in triplicate for progesterone by radioimmunoassay as described by Beltranena *et al.* (1991) using an antiserum raised against 4-pregnen-11α-ol-3, 20-dione hemisuccinate:BSA. Cross-reactions with this serum were: corticosterone 2.7%, pregnenolone 2%, 20α-progesterone 0.18%, estrone 0.12%, testosterone 0.06%, dihydrotestosterone 0.03%, cortisol 0.015%, cholesterol 0.003% and estradiol 0.002%.

Prior to assay 100 μL of plasma, diluted 1 in 10 with PBS gel buffer, was extracted with 4 mL petroleum ether for 5 min. Within each assay extraction efficiency was determined by recovery of [1,2-^{3}H(N)]-progesterone and this value was used to correct the determination of progesterone within each tube. The mean recovery of tritiated progesterone was 87.9%. Standards ranged from 0.001 to 10 ng /tube. Sensitivity of the assay, defined as 85% of total binding, was 0.02 ng/tube. No significant deviation from parallelism was apparent from assaying 100, 50 and 25 μL of a standard plasma pool. The intra- and inter-assay coefficients of variation for the two progesterone assays were 4.1% and 5.2%, respectively.

The disappearance rate constant (k - the fraction of total hormone that disappears per unit time) of progesterone after farrowing was determined by regression of the natural logarithm of plasma progesterone concentrations from during farrowing until 3 d post partum against time. The resulting regression fits the equation $X=Ae^{-kt}$ where k is the slope of the curve. Only sows for which this regression was significant at the 5% level were included in the analysis. Progesterone half life was calculated from the following equation;

$$\text{Half life (h)} = \frac{0.693}{k}$$

3.2.3 Digestibility trial

Two digestibility trials were conducted using twenty eight sows, 13 H sows (2 x parity 1, 7 x parity 2 and 4 x parity 3) and 15 N sows (3 x parity 1, 6 x parity 2 and 6 x parity 3); the first between d 95 and 100 of gestation and the second from d 2 to 7 of lactation. The twenty eight sows entered the trial as they became available in 4 replicates of 9, 7, 5 and 7 sows each, which farrowed in late February, early May, early June and late June 1994, respectively. Chromic oxide was thoroughly mixed into both gestation and lactation diets at a level of 0.5%. Sows received the chromic oxide supplemented diets from d 90 of gestation until d 7 of lactation. Housing and management were otherwise the same as for all other sows on this experiment. Feed intakes were recorded daily and faecal samples were collected *per rectum* twice daily from each sow between d 95 and 100 of gestation and between d 2 and 7 of lactation. Faecal samples were frozen immediately after collection and stored at -20°C until they were freeze-dried. Feed samples were taken from all bags of feed used during each trial period and bulked for that period. Feed samples and freeze dried faecal samples were ground through a 1 mm sieve. Faecal samples collected during gestation were bulked together for each sow, as were those collected on d 2 to 4 of lactation (period 1) and on d 5 to 7 of lactation (period 2). Feed samples and bulked faecal samples were then analysed for chromic oxide, gross energy, ash and nitrogen. Digestibilities of dry matter, crude protein and energy were calculated.

Digestibility measurements made between d 95 and 100 of gestation i.e. before the high versus normal feed intake treatments began, provided a base line level for each sow so that each was its own control. Those made in early lactation showed the residual effect of high level feeding on digestibility in the periparturient period.

3.2.4 Chemical analyses

Chromic oxide levels were determined in feed and faecal samples using the method of Fenton and Fenton (1979). Chromic oxide analyses were conducted in triplicate. Chromic oxide concentrations in the diets and faeces were calculated from the following equations:

Cr_2O_3 % in diet = (100 x (OD-0))/(1000 x (0.0129095 x wt of sample(w))

Cr_2O_3 % in faeces = (100 x (OD-0.0047083 x w))/(1000 x (0.0129095 x w))

Nitrogen content of feed and faeces was analysed using an FP-428 Nitrogen Determinator, System: 601-700-900 (LECO Corporation, St. Joseph, Michigan). Dry matter (DM) was determined by drying a known weight of sample in a drying oven overnight (AOAC, 1990). Gross energy contents were measured using an adiabatic bomb calorimeter (AOAC, 1990).

Digestibility was calculated as;

$$\text{Digestibility of X} = \frac{\%Cr_2O_3 \, / \, \text{X in faeces} - \%Cr_2O_3 \, / \, \text{X in food}}{\%Cr_2O_3 \, /\text{X in faeces}}$$

Where X = Dry matter (expressed as a percentage), crude protein (expressed as a percentage), or gross energy (expressed as kJ/g).

3.2.5 Statistical analyses

Data for sow feed intake, BW, fatness, changes in BW and fatness, pre-treatment plasma progesterone concentration, disappearance rate constant, half-life, pre-treatment digestibility, piglet number, BW and growth rate were analysed using analyses of variance. Sources of variation were treatment (high or normal feed intake during the last 2 weeks of gestation), parity (p=3), interaction between feed intake and parity and sow within feed intake by parity (error term). Although some sows were studied over more than one parity they were considered as different individuals in each parity. Preliminary analyses indicated no significant feed intake by parity interactions and therefore only main effect least square means are presented. Comparisons among parity least square means were made using Fisher's protected least significant difference (Ott, 1993). All computations were made using MANOVA operation of Systat (Wilkinson, 1990). Data for plasma progesterone concentrations during the treatment period and at farrowing were analysed according to the procedure described above plus a covariate of pre-treatment plasma progesterone concentration. Comparison between progesterone concentration of post prandial samples before treatment (d 98) and during treatment (d 109) was computed as a split-plot addition to the model given above for pre-treatment progesterone concentration. Likewise comparison between progesterone concentration of preprandial and postprandial samples during treatment (d 109) were also computed as a split-plot addition to the model given above for pre-treatment progesterone concentration.

Data for digestibility during period 1 and period 2 of lactation were analysed using analyses of variance with sources of whole plot variation of replicate ($r=4$), feed intake in late gestation ($FI = 2$), parity ($p=3$), feed intake by parity, covariate of pre-treatment digestibility, sow within replicate by feed intake by parity (error term), split plot variation of the periods ($pd = 2$) and period by feed intake, period by parity and period by feed intake by parity interactions.

Initial analyses established few treatment differences and therefore step-wise multiple regressions across the complete data set were used to study relationships between chosen parameters (Wilkinson, 1990).

3.3 Results

Please note: There were no significant interactions between treatment and parity for any of the variables measured.

3.3.1 Gestation length

There was no difference in gestation length between either treatments or parities. Overall the average gestation length was 115.6 (±0.13) days.

3.3.2 Feed intake

Daily feed intakes of H and N sows during late gestation and lactation are illustrated in Figure 3.2 and detailed in Table 3.3. From d 100 of gestation until farrowing mean daily intakes of H and N sows were 3.9 ± 0.04 and 2.3 ± 0.03 kg/d, respectively ($P < 0.001$). There was no difference in total lactation feed intake between the two treatments (6.5 ± 0.13 kg/d). H sows tended to eat less feed than N sows on the first d of lactation, 3.3 versus 4.0 kg respectively ($P < 0.08$). Feed intake increased throughout lactation with sows averaging 5.1 kg/d in wk 1, 6.6 kg/d in wk 2 and 7.5 kg/d in wk 3 of lactation.

Parity 2 and 3 sows ate more than parity 1 sows throughout lactation ($P < 0.001$), 7.1 kg/d versus 5.6 kg/d overall, respectively, although this effect was least on d 1 of lactation when feed intake of parity 3 sows was not significantly greater than that of parity 1 sows (Table 3.4). Feed intake was normally distributed within parities (Figure 3.3).

Multiple regression analysis of feed intake data showed that the mean daily feed intake during lactation increased with increasing sow parity and litter weight gain (increased milk production) and decreased with increasing sow fatness;

Equation 3.1

$$\mathbf{FI_L = \underset{(0.651)}{5.29} + \underset{(0.167)}{0.88}\,P - \underset{(0.029)}{0.08}\,B_f + \underset{(0.233)}{0.56}\,LITWG_{20}}$$

$$(R^2 = 0.383,\ P < 0.001)$$

Where; FI_L = mean daily feed intake during lactation (kg/d)

P = parity

B_f = P2 backfat thickness at farrowing (mm)

$LITWG_{20}$ = average daily litter liveweight gain (kg) to d 20

This regression was not improved by including gestation feed intake. Parity was a better predictor of feed intake than sow liveweight. Sow backfat thickness at farrowing had a significant effect on mean daily feed intake ($P < 0.004$) over and above the effect of parity but its influence on actual feed intake was small, feed intake declining by only 85 g/d for every increase of 1 mm in sow backfat thickness at farrowing.

When regressions were computed for intake in each of the first three weeks of lactation it was found that litter growth rate, an indicator of milk production, became significantly correlated to feed intake as lactation progressed and that sow fatness was no longer correlated to feed intake by week 3. Parity was an important determinant of feed intake in all weeks of lactation. Increases of 1 kg/d in feed intake from d 100 of gestation until farrowing resulted in a corresponding increase in feed intake of 0.34 kg/d in week 3 of lactation. Farrowing liveweight had a small negative effect on feed intake in week 3.

Equation 3.2 $$\mathbf{FI_{wk\,1} = \underset{(0.476)}{4.76} + \underset{(0.153)}{0.87}\,P - \underset{(0.026)}{0.08}\,B_f}$$

$(R^2 = 0.241,\ P<0.001)$

Equation 3.3 $$\mathbf{FI_{wk\,2} = \underset{(0.644)}{5.17} + \underset{(0.198)}{0.80}\,P - \underset{(0.031)}{0.08}\,B_f + \underset{(0.218)}{0.77}\,LITWG_{1\text{-}6}}$$

$(R^2 = 0.315,\ P<0.001)$

Equation 3.4 $$\mathbf{FI_{wk\,3} = \underset{(1.220)}{5.54} + \underset{(0.333)}{1.10}\,P - \underset{(0.008)}{0.02}\,LWT_f + \underset{(0.278)}{0.99}\,LITWG_{20} + \underset{(0.178)}{0.34}\,GFI}$$

$(R^2 = 0.380,\ P<0.001)$

Where; $FI_{wk\,1}$ = mean daily feed intake during wk 1 of lactation (kg/d)

$FI_{wk\,2}$ = mean daily feed intake during wk 2 of lactation (kg/d)

$FI_{wk\,3}$ = mean daily feed intake during wk 3 of lactation (kg/d)

LWT_f = liveweight at farrowing (kg)

$LITWG_{1\text{-}6}$ = litter liveweight gain from birth to d 6 (kg)

GFI = sow feed intake from d 100 of lactation until farrowing (kg/d)

3.3.3 Changes in liveweight and backfat

At the start of the experiment (d 98 of gestation) there was no difference in liveweight or backfat between the two treatment groups, mean liveweight was 201.7 ± 2.98 kg and mean P2 backfat thickness was 19.0 ± 0.44 mm. There was also no treatment difference in liveweight gain between breeding and d 98 of gestation. N sows lost an average of 3.9 kg liveweight and 1.6 mm backfat between d 98 and farrowing whilst H sows gained 3.9 kg liveweight and 0.2 mm backfat ($P < 0.001$, Figures 3.4 and 3.5, Table 3.5.). As a result H sows gained more weight during the whole of gestation than N sows ($P < 0.06$). Despite this there was no significant difference between treatments in liveweight or backfat thickness after farrowing.

Sows from both treatments lost similar amounts of backfat during lactation so that from d 98 to weaning H sows lost an average of 1.2 mm less fat than N sows ($P < 0.05$). Because H sows tended to lose more weight during lactation than N sows ($P < 0.075$), overall liveweight loss between d 98 of gestation and weaning was similar for both treatments.

Parity 1 sows gained more weight between breeding and d 98 than parity 2 or 3 sows, 59.6 ± 2.25 kg versus 52.4 ± 2.45 and 50.6 ± 2.77 kg, respectively ($P < 0.05$) but because of the treatments imposed from d 98 to farrowing there was no difference between parities in total liveweight gain during gestation (i.e. breeding to weaning, $P<0.07$). Throughout the trial parity 1 sows were lighter than parity 2 sows which were lighter than parity 3 sows ($P < 0.001$, Table 3.6). Parity 1 and 2 sows were leaner than parity 3 sows during the whole experiment. Parity 1 sows lost less backfat between d 98 and farrowing than the older sows. P2 backfat levels were normally distributed within parity (see Figure 3.6), but whereas parity 1 sows showed a tight grouping around the average value of 17.3 mm backfat, with successive parities the normal distribution curve became increasingly flatter as a greater proportion of sows varied from the mean. The variances in farrowing P2 backfat were 7.7, 10.1 and 50.5 for parities one, two and three, respectively. Despite this the distribution of backfat loss across parities was remarkably similar as shown in Figure 3.7.

Regression analysis revealed a weak negative relationship between late gestation feed intake and liveweight change of sows during lactation ($R^2 = 0.034$, $P < 0.055$), however, when overall pregnancy weight gain was put into the regression equation, gestation feed intake ceased to have any importance. Liveweight loss during lactation was greater in sows which had greater weight gain during gestation, higher liveweight at farrowing and larger litter size. Lactation weight loss was reduced in sows which had larger daily feed intakes throughout lactation and in those which were fatter at farrowing;

Equation 3.5

$$\mathbf{LWL_{f\text{-}w} = \underset{(10.524)}{-\,29.16} + \underset{(0.048)}{0.21}\ LWT_f + \underset{(0.639)}{4.36}\ LS_w - \underset{(1.106)}{6.67}\ FI_L - \underset{(0.339)}{0.98}\ B_f + \underset{(0.077)}{0.30}\ GWG}$$

$$(R^2 = 0.541,\ P < 0.001)$$

Where; $LWL_{f\text{-}w}$ = Liveweight loss from farrowing to weaning (kg)

GWG = Gestation weight gain (kg)

LS_w = litter size at weaning

It is immediately apparent that lactation weight loss was largely determined by sow feed intake and litter size. Stepwise multiple regression revealed that litter size was the single most important determinant of liveweight loss in lactation ($R^2 = 0.180$,

$P<0.001$). Farrowing liveweight and farrowing backfat thickness of the sow only became significantly correlated to lactation liveweight loss after the other variables had been incorporated into the equation. Weaning weight of piglets or any other measurement of piglet growth did not improve the regression equation.

In contrast, sow backfat loss between farrowing and weaning was related only to daily feed intake during lactation, litter size and weaning weight of piglets;

Equation 3.6

$$\mathbf{P2L_{f\text{-}w} = -\underset{(1.409)}{2.51} - \underset{(0.162)}{0.81}\,FI_{L} + \underset{(0.098)}{0.67}\,LS_{w} + \underset{(0.149)}{0.60}\,PWT_{w}}$$

($R^2 = 0.434$, $P<0.001$)

Where; $P2L_{f\text{-}w}$ = sow backfat loss during lactation (mm).

PWT_w = average piglet weaning weight (kg).

Litter size was the most significant determinant of backfat loss accounting for 50% of the explained variance ($R^2 = 0.228$, $P<0.001$). Feed intake and piglet weaning weight only became significant factors after litter size had been incorporated into the regression equation.

Sows which had 6 mm or more backfat loss during lactation were compared to those which gained backfat thickness during lactation. There was no difference between the two groups in terms of liveweight and backfat thickness at farrowing but sows which gained backfat during lactation reared smaller litters (5.8 ± 0.47) than sows with high fat loss (9.6 ± 0.47, $P < 0.001$). Surprisingly there was no difference in feed intake during lactation or piglet growth rates between the two groups but high fat loss sows also lost more liveweight during lactation ($P < 0.03$).

3.3.4 Plasma progesterone

There was no difference between plasma progesterone concentrations of H and N sows at any stage measured in late gestation or early lactation, nor in the decline in progesterone in the first 6 h after farrowing (see Table 3.7 and Figure 3.8). Pre-treatment plasma progesterone concentrations on d 98 of gestation varied considerably between sows and were determined to some extent by sow liveweight, with plasma progesterone concentrations increasing in heavier sows ($R^2 = 0.221$, $P < 0.001$). Plasma progesterone on d 98 of gestation was not related to the number of piglets in the litter.

On d 109 of gestation, progesterone concentrations were similar before and after feeding. Plasma progesterone concentrations of high intake sows did not decline between d 98 and 109 of gestation in response to increased feeding and were not different from those of normal intake sows. Progesterone concentrations at farrowing were best predicted from the inherent progesterone concentration of the sow (on d 98 of gestation) and the total number of piglets born;

Equation 3.7 $$\mathbf{P4_f = -\underset{(2.05)}{0.57} + \underset{(0.086)}{0.23}\,P4_{d98} + \underset{(0.122)}{0.28}\,LS_b}$$

($R^2 = 0.363$, $P < 0.001$)

Where; $P4_f$ = sow plasma progesterone concentration at farrowing (ng/mL).

$P4_{d98}$ = sow plasma progesterone concentration on d 98 of gestation (ng/mL).

LS_b = total number of piglets born.

Disappearance rate constants could only be calculated for 34 of the 44 sows and were extremely variable between individual animals and not different between treatments. Progesterone half life averaged 41.2 ± 3.81 h. Regression analysis identified a positive relationship between disappearance rate constant (k) and plasma progesterone at farrowing but a negative relationship to litter size at birth and to sow backfat thickness at farrowing. Therefore, half life was shorter when progesterone at farrowing was higher, but became longer as the number of piglets in the litter, or the fatness of the sow at farrowing, increased;

Equation 3.8 $$\mathbf{k = \underset{(0.010)}{0.047} + \underset{(0.001)}{0.002}\ P4_f - \underset{(0.001)}{0.002}\ B_f - \underset{(0.001)}{0.001}\ LS_b}$$

$(R^2 = 0.401,\ P < 0.01)$

Where; k = progesterone disappearance rate constant.

Sow backfat thickness at farrowing and inherent plasma progesterone concentration of the sow had the most significant influence on the disappearance rate constant. The disappearance rate constant is used to calculate half life of plasma progesterone. Since this is an inverse relationship the smaller the value of k, the longer the half life.

There was no relationship between progesterone concentrations during and after farrowing, nor the rate of decline of progesterone, with piglet weight gain in the first two d of life. There was a significant although weak correlation between decline in progesterone in the first 6 h after birth and piglet growth rate from birth to six d of age

($R^2 = 0.109$, $P < 0.05$). Inclusion of litter size did not improve the regression. The disappearance rate constant was not correlated to any measure of piglet growth.

On d 98 of gestation parity 2 and 3 sows had significantly higher plasma progesterone concentrations than parity 1 sows, 25.7 and 27.7 ng/mL versus 21.9 ng/mL ($P<0.05$, Table 3.8), respectively. The increased plasma progesterone concentration appears to be due to the greater liveweight of older sows. Although parity was correlated to plasma progesterone concentration on d 98 of gestation ($R^2 = 0.159$, $P < 0.01$), regression analysis showed that liveweight accounted for more of the variation in this parameter and that parity was not significant once liveweight had been accounted for.

3.3.5 Piglet performance

Piglet birthweight was not affected by feed intake in late gestation and there were no significant differences in any other aspect of piglet performance between the two treatment groups (see Table 3.9). Sows produced an average of 11.0 ± 0.27 piglets per litter of which 0.6 ± 0.12 were stillborn. After cross-fostering to balance litter size, sows had an average of 9.4 ± 0.17 piglets. Weight gain in the first 2 d of life averaged 113 ± 9.0 g/d per piglet, 244 ± 4.2 g/d over the first 20 d and 257 ± 5.1 g/d to weaning. Sows weaned an average of 8.5 ± 0.19 piglets with a mean weight of 7.8 ± 0.14 kg.

Parity 1 sows produced smaller litters of lighter piglets than higher parity sows (litter size was not significantly different between parities 1 and 2; Table 3.10) and this effect remained even when farrowing liveweight was used as a covariate. Parity 1 and 2 sows had less stillborn piglets than parity 3 sows, 4% of all piglets born versus 10% for parity 3 sows. After cross-fostering all three parities had similar litter size. There was no difference between parities in piglet growth rate in the first two days of life, nor in preweaning mortality or the number of piglets weaned. Whilst there was a significant difference in growth rate of piglets from sows of different parities in the first six days of life, this was not significant when piglet birthweight was used as a covariate. Overall piglets from parity 1 sows grew more slowly than those from later parities and therefore were lighter at weaning, 6.9 kg versus 8.5 kg for parities 2 and 3. Piglet birthweight remained a significant covariate for piglet growth throughout lactation ($P < 0.001$) but parity also had a significant effect on piglet growth ($P < 0.05$) beyond 6 d of age.

Regression analysis showed that litter weight at birth was negatively correlated to sow fatness at farrowing and positively correlated to mean plasma progesterone concentration between d 98 and d 109 of gestation, sow liveweight at farrowing and number of piglets born;

Equation 3.9 $$\mathbf{TPWT_b = \underset{(2.140)}{-0.26} + \underset{(0.075)}{0.26}\, P4_{pre\text{-}f} + \underset{(0.013)}{0.04}\, LWT_f - \underset{(0.123)}{0.51}\, B_f + \underset{(0.097)}{0.91}\, LS_b}$$

($R^2 = 0.889$, $P < 0.001$)

Where; $TPWT_b$ = litter weight at birth.

$P4_{pre\text{-}f}$ = mean plasma progesterone concentration d 98 to 109 of gestation (ng/mL).

Litter size and mean plasma progesterone concentration between d 98 and 109 of gestation were the parameters most highly correlated with litter birthweight ($R^2 = 0.820$, $P<0.001$), with sow liveweight and fatness contributing little more to the variance.

The following regression equation for mean piglet birthweight was computed for all sows in the study. Plasma progesterone concentrations were not available for all sows (see Materials and Methods) and therefore have not been included in this regression;

Equation 3.10

$$\mathbf{PWT_b = -\underset{(0.156)}{1.45} + \underset{(0.001)}{0.004}\,LWT_f - \underset{(0.005)}{0.02}\,B_f - \underset{(0.008)}{0.05}\,LS_b + \underset{(0.001)}{0.003}\,GWG - \underset{(0.002)}{0.008}\,LGWG}$$

($R^2 = 0.386$, $P<0.001$)

Where; PWT_b = mean piglet birthweight (kg)

LGWG = sow liveweight gain between d 98 of gestation and farrowing (kg)

Litter size and sow backfat thickness at farrowing were the variables most closely correlated to piglet birthweight. Farrowing liveweight only became a significant variable when all the other variables had been included in the regression. Surprisingly gestation weight gain and weight gain between d 98 and farrowing had opposing effects on piglet birthweight.

It was not possible to obtain meaningful regressions for piglet liveweight gains in the first two days of life. Daily liveweight gain to 6 d of age was related to piglet birthweight which was the most significant determinant of growth rate in this period with piglet growth rate increasing by 52 g/d for each kg birthweight. The range in average birthweight in this experiment was between 800 and 2200 g which would have translated to a growth rate difference of 72.8 g/d. Daily liveweight gain to 6 d of age increased with each successive parity of the sow and decreased with increasing litter size, but this latter effect was quite marginal amounting to only 5 g/d/extra pig. Altogether these variables explained little of the variance in liveweight gain in the first 6 d of life.

Equation 3.11
$$\mathbf{PWG_{1\text{-}6} = -\underset{(0.047)}{0.12} + \underset{(0.021)}{0.05}\,PWT_b + \underset{(0.007)}{0.02}\,P - \underset{(0.003)}{0.005}\,LS_{d2}}$$
$(R^2 = 0.197, P < 0.001)$

Where; $PWG_{1\text{-}6}$ = average daily piglet liveweight gain to 6 d of age (kg)

LS_{d2} = litter size on d 2 of lactation

Regression analysis of daily piglet liveweight gain to 20 d of age (prior to the start of creep feeding) indicated that 35% of the variance was accounted for by piglet birthweight and sow parity;

Equation 3.12
$$\mathbf{PWG_{20} = \underset{(0.019)}{0.123} + \underset{(0.013)}{0.059}\,PWT_b + \underset{(0.004)}{0.018}\,P}$$
$(R^2 = 0.352, P < 0.001)$

Inclusion of litter size improved the R^2 of the regression;

Equation 3.13
$$\mathbf{PWG_{20} = \underset{(0.024)}{0.169} + \underset{(0.011)}{0.059}\,PWT_b + \underset{(0.004)}{0.013}\,P - \underset{(0.002)}{0.006}\,LS}$$
$(R^2 = 0.430, P < 0.001)$

Inclusion of the variables farrowing to weaning backfat loss and daily feed intake in the first 20 d of lactation explained only a further 1% of the variance.

Equation 3.14

$$\mathbf{PWG_{20} = \underset{(0.034)}{0.16} + \underset{(0.017)}{0.03}\,PWT_b + \underset{(0.005)}{0.015}\,P - \underset{(0.002)}{0.007}\,LS + \underset{(0.002)}{0.006}\,P2L_{f\text{-}w} + \underset{(0.003)}{0.009}\,FI_{1\text{-}20}}$$

$(R^2 = 0.440, P < 0.001)$

Where; PWG_{20} = average daily piglet liveweight gain to 20 d of age (kg).

LS_{20} = litter size at 20 d of age.

$P2L_{f\text{-}w}$ = loss of P2 backfat in lactation (mm).

$FI_{1\text{-}20}$ = sow mean daily feed intake for the first 20 d of lactation (kg/d).

3.3.6 Agalactia

There was no clinical incidence of agalactia in this experiment. Nine sows had litters which averaged zero or negative weight gains in the first two days of life: Of these, five were H sows (2 x parity 1, 1 x parity 2 and 2 x parity 3) and the remaining four were N sows (2 x parity 1, 1 x parity 2 and 1 x parity 3). These sows were not characterised by low feed intake, high temperature or poor subsequent litter growth rates.

3.3.7 Digestibility

Diet digestibility for H and N sows are shown in Table 3.11. There was no difference in digestibility between the treatment groups in early lactation. Pre-treatment digestibility in late gestation did not differ between treatment groups.

Dry matter digestibility averaged 78.0% ± 0.42 between d 2 and 4 of lactation and 76.8% ± 0.45 between d 5 and 7 of lactation. Corresponding digestibility coefficients over the same time periods were 80.4% ± 0.61 and 78.8% ± 0.68 for crude protein and 78.2% ± 0.46 and 77.0% ± 0.48 for energy. There was no difference between the two time periods.

3.3.8 Subsequent reproductive performance

There was no difference in the subsequent reproductive performance of sows regardless of how they were fed in late gestation (Table 3.12). Sows gave birth to an average of 12.1 ± 0.32 piglets in their next litter following an average weaning to estrus interval of 5.4 ± 0.47 d. There was also no difference in weaning to estrus interval between parities (Table 3.13). Subsequent litter size of parity 1 sows was about 1.7 piglets smaller than that of higher parity sows ($P < 0.05$).

Regression analysis found no relationship between any of the parameters measured and weaning to estrus interval. Subsequent litter size was positively correlated with feed intake in the third week of lactation, parity, sow weight gain during the previous gestation and weaning weight of the sow. However regression analysis showed that feed intake in the third week of lactation was the major influence on subsequent litter size with numbers born increasing by more than half a pig for each extra kg/d of feed intake during week 3 of lactation. Parity and sow weight gain during the previous gestation were also significant predictors of subsequent litter size. Subsequent litter size increased by almost one pig with each increase in parity between parities one and three. Gestation weight gain in the previous parity had the least effect with an increase of 25 kg required to produce one extra piglet in the subsequent litter;

Equation 3.15
$$\mathbf{SLS} = \underset{(1.973)}{\mathbf{3.09}} + \underset{(0.034)}{\mathbf{0.65}}\ \mathbf{FI_{wk3}} + \underset{(0.460)}{\mathbf{0.96}}\ \mathbf{P} + \underset{(0.018)}{\mathbf{0.04}}\ \mathbf{GWG}$$
$$(R^2 = 0.275,\ P < 0.001)$$

Where; SLS = Subsequent litter size.

Feed intakes in weeks 1 and 2 of lactation were not correlated with subsequent litter size, nor were liveweight and backfat changes in the sow during lactation.

3.4 Discussion

3.4.1 Changes in liveweight and fatness

Sows which received increased feed intake during the last two weeks of gestation did not lose backfat prior to farrowing. In contrast, the sows which remained on their normal gestation feed intake did lose backfat during the last two weeks of gestation. Both groups of sows lost similar P2 backfat thickness during lactation and therefore the advantage accrued by the sows receiving high intakes prior to farrowing was maintained through to weaning. It is generally recognised that sows which are able to maintain body condition between parities have a longer reproductive life (Whittemore, 1993). Therefore, although preservation of backfat did not confer an immediate advantage in the subsequent litter it is likely that maintenance of sow backfat thickness in the long term would increase sow longevity in the breeding herd.

Varying the feeding levels of sows for the last 16 d of gestation produced different metabolic states at farrowing. H sows were in an anabolic state having gained both backfat thickness and liveweight during this period, whilst N sows were catabolic having lost both backfat thickness and liveweight. Nevertheless, metabolic state at farrowing did not affect the ability of sows to adapt to lactation as there was no difference in lactation performance between treatments, nor in subsequent reproductive performance.

It should be remembered that all sows in this experiment were fed *ad libitum* throughout lactation and had similar liveweights and body fatness at farrowing across treatments. There were no extremely low feed intakes or dramatically large changes in liveweight and fatness during lactation as have been previously reported in experiments where lactation feed intake was deliberately restricted to generate just such effects (e.g. King and Williams, 1984ab; King and Dunkin, 1986; Eastham *et al.*, 1988; Baidoo *et al.*, 1992ab; Hughes, 1993). Regression equations from this experiment, therefore, predict significant variables for sows which were encouraged to maximise their feed intake throughout lactation.

As we might expect, fat loss between farrowing and weaning was greater in sows with larger, faster growing litters and was reduced when feed intake in lactation increased (see Equation 3.6). Interestingly fat loss in lactation was not influenced by P2 backfat at farrowing in contrast to the results of Whittemore *et al.* (1988), Yang *et al.* (1989) and Mullan and Williams (1989) who found that fatter animals at farrowing lost more fat in lactation. Although this effect was often confounded in sows fed *ad libitum* during lactation because fatter sows reduced their feed intake (Mullan and Williams, 1989), the effect did persist even when feed intakes were similar (Whittemore *et al.*, 1988). In the current experiment there was a marginal decrease in *ad libitum* feed intake with increasing sow fatness at farrowing but this did not result in greater fat loss during lactation. With this exception the regression based on the current data contained similar variables to that of Yang *et al.* (1989).

Sows which received high feed intakes in late gestation gained more liveweight prior to farrowing than flat rate fed sows but lost more liveweight during lactation, so that overall there was no difference in liveweight change between the two treatment groups. This is in agreement with the findings of Cromwell *et al.* (1989). Some of the difference in liveweight change seen at farrowing may have been due to increased gut fill resulting from greater feed intake in late gestation. This might explain the apparent relationship between gestation feed intake and liveweight loss between farrowing and weaning, since at weaning gut fills should have been similar due to similar intakes in lactation. Increased gut fill at farrowing for the H sows would also help to explain why overall gestation weight gain and weight gain between d 98 of gestation and farrowing had opposing effects on piglet birthweight. If part of the increase in liveweight between d 98 of gestation and farrowing was due to greater gut fill then the actual weight gain of the sow would be overestimated and actual piglet birthweights would be less than would be predicted from overall gestation gain.

Like backfat loss, liveweight loss during lactation increased with increasing piglet demands and this effect was reduced by increased feed intake during lactation (see Equation 3.5). However, liveweight loss was also positively correlated with liveweight at farrowing and gestation weight gain of the sow. Other workers have reported positive correlations between liveweight loss and farrowing liveweight (Yang *et al.*, 1989; Sterning *et al.*, 1990; Rojkittikhun *et al.*, 1992; Hughes, 1993) and between gestation weight gain and lactation weight loss (Mullan and Williams, 1989; Yang *et al.*, 1989). It seems that sows with greater body reserves lose them more readily during lactation. In the current experiment the regression equations for liveweight and backfat losses (Equations 3.5 and 3.6) explained only 54 and 43 % of the variance, respectively. The unexplained variance in each of these parameters is presumably due to inherent variations in metabolism between sows. It is recognised that sows of similar parity and body size fed in a standard way, with a feed allowance for sow maintenance requirement and for each piglet in the litter, will lose widely different amounts of body reserves (Whittemore *et al.*, 1980; Sterning *et al.* 1990). Part of this variance is certainly due to differences in potential piglet growth rates. Even within litters of similar size, piglet demands may vary widely depending on their birthweight (Hartmann *et al.*, 1962; Hemsworth *et al.*, 1976; van der Steen and de Groot, 1992), so that giving a standard per piglet allowance will inevitably result in disparate weight loss between sows. In the current experiment, however, variations in piglet birthweight have been accounted for by including both piglet weight and litter size in the prediction equation for backfat loss in lactation, yet there is still considerable variance between sows which remains unaccounted for. By studying the distribution of backfat thickness in sows of parities one to three (see Figure 3.6), it is evident that sow body composition at farrowing becomes increasingly diverse with successive parities. The level and range of backfat loss within different parities remained remarkably similar (see Figure 3.7). It is possible that individual sows follow the same inherent pattern of backfat loss in successive parities. In this case the effect would be cumulative so that sow backfat levels would increasingly diverge from the mean in successive parities, as observed here.

Rojkittikhun *et al.* (1992) attempted to elucidate the mechanisms underlying inherent differences between sows. They observed that prolactin levels were elevated in high weight loss sows (weight loss >25 kg over a 5 week lactation) compared to medium (11- 25 kg) and low (≤ 10 kg) weight loss animals. Unfortunately piglet weight gains were not reported in this experiment so it is not clear whether this was translated into increased milk production. However, our own results suggest that increased weight loss during lactation could result from increased milk production and this effect would be even more pronounced in sows fed a set feed allowance. This of course leaves us with a chicken and egg scenario; did the high weight loss sows have intrinsically higher prolactin so that they produced more milk and therefore heavier and more demanding piglets with resultant increased body weight loss, or did the high weight loss sows give birth to heavier piglets which stimulated increased prolactin secretion and concurrent increased milk production? High weight loss sows in Rojkittikhun *et al.*'s (1992) experiment were heavier at farrowing and in the current experiment such sows produced heavier birthweight piglets (Equation 3. 10) which stimulated greater milk production (see Equations 3.12 to 3.14). Unfortunately the lack of piglet data in Rojkittikhun *et al.*'s (1992) experiment makes it impossible to resolve this issue. Other aspects of inherent metabolic differences between sows remain to be elucidated.

3.4.2 Lactation feed intake and digestibility

It is widely recognised that longer term increases in gestation feeding will adversely affect lactational appetite (e.g. Salmon-Legagneur and Rerat, 1962; Mullan and Williams, 1989; Weldon *et al.*, 1994). However, increased feed intake during the last two weeks of gestation did not decrease feed intake in lactation proving that short term increases in feed intake at a time when sows would otherwise be catabolic is not detrimental to subsequent lactation feed intake. This is in agreement with the work of Cromwell *et al.* (1989) and Stirling and Cline (1986) who also found no deleterious effect on lactation feed intake from increasing feeding level in late gestation. The average overall gestation feed intake for H sows was approximately 26 kg greater than that of N sows, this is equivalent to an overall increase of 9.6 % in total gestation feed intake. For every increase of 4.2 MJ ME/d in gestation feed intake there is a corresponding decrease in lactation feed intake of 2.1 MJ ME/d (Aherne and Williams, 1992). Therefore averaged across gestation H sows received an extra 2.9 MJ DE/d which should correspond to a decline in lactation feed intake of 1.3 MJ DE/d, or approximately 2.6 kg/sow (0.1 kg feed/sow/d) over the whole of lactation. In view of the large variation in lactation feed intake between sows, it is therefore not surprising that no significant decline in feed intake was observed. Indeed in this experiment, there was no trend towards decreased feed intake in the H sows, if anything the opposite was true since there was a positive correlation between feeding level in late gestation and feed intake in the third week of lactation. Clearly increasing feed intake in the last two weeks of gestation can be used to improve sow body condition without risking a drop in lactation feed intake.

Although increased feed intake to H sows was only supplied during the last two weeks of gestation it is appropriate to consider the increase across the whole of pregnancy. Elsley *et al.* (1971) observed with 154 sows over 3 parities at seven research centres that pattern of feed intake during pregnancy had no effect on sow liveweight at farrowing or on any aspect of sow performance. Sows within each treatment received the same overall gestational feed intake but they were fed according to one of the following patterns; 1) constant - flat rate of 1.9 kg/d throughout, 2) low-high - feed intake increasing from 1.4 kg/d in early gestation to 2.5 kg/d in late gestation, 3) high-low - feed intake declining from 2.5 kg/d in early gestation to 1.8 kg/d in late gestation, and 4) high-low-high - feed intakes of 2.5 kg/d in early and late gestation with 1.4 kg/d in the middle. In contrast to Elsley's findings, more recent work suggests that there may be crucial periods during gestation when feed intake may have profound effects on sow and litter performance. For example, Dyck *et al.* (1980), Ashworth (1991) and more recently Jindal *et al.* (1996) suggest that high feed intake in very early gestation adversely affects embryo survival. It has also been suggested that high feed intakes between d 75 and 90 of lactation may compromise differentiation and growth of secretory tissue in the mammary gland (Head and Williams, 1991; Head, *et al.*, 1991; Weldon *et al.*, 1991) resulting in reduced lactation performance (Head and Williams, 1991). However this hypothesis is not supported by the work of Elsley *et al.* (1971), described earlier, in which there was no difference in piglet performance between sows fed high intakes at different stages of gestation, nor by that of Weldon *et al.* (1994) who fed gilts *ad libitum* (3.7 kg/d) or 1.85 kg/d from d 60 of gestation and reported no difference in piglet growth rates between treatments. In contrast, increasing sow feed intake between d 25 to 50 of gestation increases the number of secondary muscle fibres formed in fetuses and may result in improved growth rates during the growing and finishing periods (Dwyer *et al.*, 1994).

Lactation feed intake in this experiment compared favourably to other values reported in the literature with an overall daily mean intake of 6.6 kg. Cromwell *et al.* (1989) reported a mean lactation feed intake through 21 d of 5.7 kg/d for multiparous sows, whilst Weedon *et al.* (1994) obtained intakes averaging between 5.1 and 5.5 kg/d in parity 2 sows. Mean intakes of primiparous sows in the current experiment averaged 5.6 kg/d and are higher than other reported values for primiparous sows which range from 4.5 to 4.7 kg/d (King and Williams, 1984a; Koketsu, 1994; Weedon *et al.*, 1994). As a result of these high feed intakes, liveweight and backfat losses during lactation were moderate compared to other values reported in the literature.

The overall equation for daily feed intake throughout lactation obtained in this experiment (Equation 3.1) was similar to that of O'Grady *et al.* (1985) based on an analysis of 3,559 lactations. However, they found that, in addition to sow parity and litter demands, defined in their equation by litter size and lactation length, pregnancy weight gain was also an important factor. In the current experiment pregnancy weight gain was not correlated to daily feed intake in lactation and did not explain any more of the variance than the parameters already described. Despite their much larger sample size and use of a more complex regression equation, the amount of variance explained by the O'Grady equation is considerably less than that explained by our equation, $r = 0.49$ versus $r = 0.61$, accounting for 24% and 38% of the variance, respectively. This difference is almost certainly due to the use of varying feeds and feeding systems throughout the seven years of the O'Grady study which would have contributed to variation in sow feed intake. In our experiment all sows were offered similar feed under similar management conditions. Koketsu (1994), in an analysis of 25,040 records of sow lactation feed intakes from 30 commercial farms, agreed that average daily feed intake is affected by the parity of the sow and her level of milk production, in this case a function of weaning weight and lactation length. In this large study he found that average high temperature in the farrowing room, energy density of the diet and pattern of feed intake of the sow in lactation were also important. In addition there were differences between the farms studied.

It is widely accepted that feed intake in lactation is negatively correlated to sow backfat thickness at farrowing (O'Grady *et al.*, 1985; Matzat *et al.*, 1990a; Mullan and Williams, 1989). In this experiment sow condition at farrowing did indeed negatively affect feed intake but this was a small effect, amounting to a reduction in feed intake of 85 g/d for every increase of 1 mm in P2 backfat, which was only apparent for the first 2 weeks of lactation (see Equations 3.2 and 3.3). Thereafter the demands for milk production, i.e. litter size and piglet growth rate, determined sow feed intake (Equation 3.4). Revell *et al.* (1994) also found that voluntary feed intake of sows was only affected by the sow backfat thickness at farrowing in the first two weeks in lactation. Although in their experiment feed intake after the first two weeks of lactation depended on the protein concentration in the diet with greater intakes of the high (19%) protein diet than of the low (7.9%) protein diet. More recently Revell *et al.* (1995) observed that fatter sows ate consistently less than thinner sows throughout lactation and that this was correlated to higher preprandial plasma concentrations of glycerol and non-esterified fatty acids (NEFA) in the circulation. There was no corresponding difference in insulin or glucose concentrations. They postulated that NEFA and glycerol concentrations in plasma may be the signal via which body fatness regulates voluntary feed intake in lactating sows.

If feed intake in early lactation is determined by factors other than milk production then sows with high milk outputs will suffer greater loss of body reserves and will therefore be more catabolic at this stage. If sows are weaned at this point then subsequent reproductive performance may be adversely affected. Lack of ability of sows to respond to milk output demands in early lactation may explain why this period is important for subsequent reproductive performance even in conventionally weaned sows (Koketsu *et al.*, 1994).

Whilst increased feed intake in late gestation did not decrease lactational feed intake neither did it cause an increase in feed intake in early lactation, as was suggested in the hypotheses of this experiment. Although it is likely that the gastrointestinal tract and its associated organs would have shown some enlargement in response to increased feed intake over the last 16 d of gestation, as was seen with gilts following 16 d of high intake feeding in Chapter 2, this was not reflected in increased feed intake in early lactation, nor in increased diet digestibility at this time.

3.4.3 Feed intake and milk yield

Parity 1 sows ate consistently less than sows of parities 2 and 3 throughout lactation. This was true both in absolute terms and in terms of metabolic requirements. When the sow's maintenance energy requirements had been satisfied (460 kJ DE/kg$^{0.75}$, NRC, 1988), parity 1 sows had 55.6 ± 2.46 MJ DE/d (average across lactation) remaining for milk production compared to 71.3 ± 2.81 and 70.8 ± 2.15 MJ DE/d for sows of parities 2 and 3 respectively. Likewise for protein intake; maintenance requirement of sows for protein was calculated using Everts' (1994) value of 2.81 g protein/kg$^{0.75}$. Parity 1 sows had only 729.8 ± 28.00 g protein/d remaining for milk production once sow maintenance requirement had been met, versus 915.7 ± 31.37 and 943.1 ± 36.46 g protein/d for parity 2 and 3 sows, respectively. Since litter size and loss of body reserves were similar between sows of different parities this explains the reduced growth rate of piglets from parity 1 sows. While a constant value has been used in this thesis to calculate the protein requirement for maintenance in lactating sows (Everts, 1994), it is by no means certain that maintenance requirement for protein would remain constant between sows at different stages of maturity facing varying degrees of metabolic demands. Millward (personal communication) has determined that maintenance requirements for protein are significantly higher on a high protein intake than on a low protein intake. In the current experiment parity 1 sows ate less than later parity sows and consequently also had lower protein intakes. It is therefore possible that their maintenance requirements for protein were also lower, thus sparing a greater proportion of dietary protein for milk production. However, even if this was the case, parity 1 sows would still have had less dietary protein available for milk production than the later parity sows.

Millward's observation that the level of protein intake determines the maintenance requirement for protein may also help to explain why lighter sows at farrowing lost less weight during lactation (Equation 3.5). If such sows have a reduced maintenance requirement for protein then they will use both energy and protein more efficiently and hence should conserve more of their body reserves.

It is interesting to speculate whether first parity sows have an inherently lower capacity for milk production than older sows. In this experiment, parity 1 sows gave birth to lighter piglets than did older sows and had significantly lower piglet growth rates throughout lactation. When piglet birthweight was used as a covariate for piglet growth rate in the first six days of life there was no difference in growth rates between piglets from sows of different parities. However, as lactation progressed differences in piglet size at birth were no longer sufficient to explain the differences in milk production between sows of different parity. It is clear from many other experiments (e.g. Mullan and Williams, 1989; Yang *et al.*, 1989) that first parity sows have the capacity to mobilise considerably greater amounts of body reserves than they did in the current experiment. Therefore it appears that the gilts in this experiment had an inherently lower capacity for milk production than the older sows. This observation is supported by the data of Pluske *et al.* (1995) who found that when gilts were 'superalimented' by force-feeding them via a stomach cannula so that they received an extra 29 MJ ME/d above the intake of *ad libitum* fed controls, there was no corresponding increase in milk production. Although further analysis of the same data set by Clowes *et al.*(unpublished), investigating only sows with at least eight piglets per litter, determined that superalimented sows did produce faster growing piglets than did *ad libitum* fed controls but that the increase in growth rate was lower than would be predicted from the sows' feed intake. Instead the gilts converted the extra feed into maternal gain. In contrast, Matzat *et al.* (1990b) used the superalimentation technique with multiparous sows and established a linear relationship between milk production and maternal feed intake. Clearly there is a difference in the potential milk output of gilts versus sows, although it has been suggested that the explanation may simply be that gilts have less secretory cells in mammary tissue than sows (Williams, 1995). In the present experiment piglet growth rate was closely related to piglet birthweight with parity 1 sows giving birth to smaller piglets. Since piglet demand is one of the primary determinants of milk output (Auldist and King, 1995) parity 1 sows may produce less milk than later parity sows because they receive less stimulation from their smaller piglets.

3.4.4 Piglet birthweight

Piglet birthweight was not increased by high level feeding in late gestation. This is in agreement with the results of Hillyer and Phillips (1980), Pond *et al.* (1981) and Sterling and Cline (1986). In contrast, Cromwell *et al.* (1989) obtained an increase of 39 g in piglet birthweight in response to high intake feeding in late gestation but this was only seen after sows had been on the treatment for two or more gestation-lactation cycles. Their sows increased in liveweight as they remained on the high intake treatment through successive parities and hence the increase in birthweight may have been caused by the increased liveweight of the sows rather than by high feeding level in late gestation *per se*. We have shown in this trial that piglet birthweight is positively correlated to sow liveweight.

In the current experiment, litter weight at birth was greater in sows of heavier liveweight but lower in fatter sows suggesting that this parameter is more closely related to the lean body size of the sow rather than to her absolute body size (see Equations 3.9 and 3.10). Changes in sow fatness had a greater effect on litter weight at birth than changes in sow liveweight. One kg difference in litter weight at birth resulted from 2 mm change in backfat versus 23 kg change in liveweight. Mullan and Williams (1989) found that heavier piglets were born to gilts fed a high feed intake in gestation (3.0 kg/d from conception to d 84 of gestation, followed by 2.0 kg/d until farrowing) than to those fed a low intake in gestation (1.5 kg/d throughout). The high intake gilts were significantly heavier and fatter at farrowing. It was not clear whether the increased birthweight of the piglets was due to the larger size of the sow in late gestation or to her higher feed intake in gestation. Gilts which received 2.0 kg/d throughout gestation (medium intake) gave birth to lighter piglets than the high intake gilts even though feed intake levels had been similar between d 84 and farrowing. Mullan and Williams (1989) suggested that either increased feed intake in early gestation causes increased placental size so that the advantage is maintained through to farrowing or that the high intake gilts were able to use their body reserves for fetal growth in late gestation. The latter explanation seems less likely since the medium intake gilts also had substantial body reserves at farrowing and yet did not grow their piglets equivalently. Therefore it could be that the larger size of the high intake gilts provided extra uterine and placental space.

3.4.5 Plasma progesterone concentration

In Chapter 2 a significant increase in MCR of progesterone was observed, resulting in lower plasma progesterone concentrations, in response to doubling feed intake of ovariectomised gilts. It might therefore be expected that a comparable reduction in plasma progesterone levels in high intake sows between pre-treatment values and those on d 109 of gestation, particularly in comparison to the levels in N sows would be seen. Surprisingly this was not observed. Instead there was a trend towards an increased progesterone concentration on d 109 in the H sows. It seems clear from the work described in Chapter 2 and that of others (Prime and Symonds, 1993) that there is an increase in clearance rate of progesterone in response to increased feeding level in gilts. It is thought that this results primarily from an increase in blood flow through the liver which is the most efficient organ of progesterone extraction in the body. If the same mechanism occurred in pregnant sows in the current experiment and clearance rate of progesterone was increased in our higher intake animals then there must have been a corresponding increase in production rate since there was no decline in plasma progesterone concentration. This could occur as the result of negative feedback, although this was not observed in studies with rats (Waddell *et al.*, 1982), or through increased hormonal stimulation. Both insulin and IGF-1 have been shown to increase progesterone secretion in a variety of species (Braden *et al.*, 1994) and both increase with increasing plane of nutrition. Therefore, high intake feeding of sows in late gestation may well result in increased progesterone production as well as increased clearance. However, it is equally likely that no change in plasma progesterone concentration was observed because there was no change in clearance rate in response to increased feed intake in pregnant sows.

In the ovariectomised gilt, progesterone levels decreased postprandially in response to increased clearance, presumably via increased splanchnic blood flow in response to feed intake. The sows in this experiment were fed only once daily and therefore feeding might be expected to have a dramatic effect on progesterone clearance rate with a resulting depression in plasma progesterone concentrations. This was not observed and again there are two possible explanations. Either there was no increased clearance in response to feeding or secretion of progesterone increased proportionally, perhaps in response to the feeding-induced surge of insulin.

Plasma progesterone concentration in late gestation was not related to resultant litter size but plasma progesterone concentration during farrowing was positively correlated to this parameter. This suggests that there was some residual progesterone production at farrowing related to litter size. This is almost certainly due to feto-placental production (Craig, 1982) which will be directly related to fetal number and placental mass. The disappearance rate constant of progesterone and its half life in the circulation of sows following farrowing were of the same order of magnitude as those measured in ovariectomised gilts (Chapter 2). The disappearance rate constant was positively correlated to plasma progesterone concentration during farrowing. Therefore, the lower the plasma progesterone concentration during farrowing, the lower the disappearance rate constant and the longer the half life of progesterone. This supports the observations made in Chapter 2 that prolonged half lives were associated with lower plasma progesterone concentrations. There are many progesterone pools in the body, including adipose tissue and blood, each one of which will have its own clearance rate for progesterone so that overall disappearance rate of progesterone is under multi-factorial control. One factor which may be important as progesterone concentrations decline is the ratio of progesterone to cortisol binding globulin. This will be higher so that a greater proportion of progesterone may be bound thus making it less available for extraction from the blood.

In this experiment disappearance rate constant was negatively related to sow backfat thickness and therefore fatter sows had longer half lives than thinner sows. This is in contrast to the work with ovariectomised gilts (Chapter 2) where body condition did not affect half life. Progesterone is lipophilic in nature. Hillbrand and Elsaesser (1983) found that the concentration of progesterone in adipose tissue could be more than 200 times greater than that in plasma. If we assume that there is an equilibrium between progesterone concentrations in plasma and those in fat then, following 115 d of elevated plasma progesterone in pregnancy, we would expect progesterone concentrations in fat to be high and, within different fat stores such as backfat and kidney fat, similar per unit of fat. In this case fatter sows would have a greater total body content of progesterone than thinner sows because of their greater proportion of fat. Hence we might expect progesterone to be released at a greater rate in fatter sows than in thinner, and possibly over a longer time period, thus sustaining higher levels of progesterone in plasma following farrowing. Progesterone would therefore have a longer half life in such animals. This is not in agreement with the findings of Chapter 2 or those of Hillbrand and Elsaesser (1983) who reported that, after ovariectomy or progesterone injection, gilts showed no clear effect of the fat to lean ratio on changes in backfat or plasma progesterone concentrations during the estrous cycle. It is, however, in line with one of the hypotheses proposed in Chapter 2 regarding disappearance rate of progesterone following removal of the progesterone source, and makes biological sense.

Disappearance rate constant was also negatively correlated to litter size at birth. This can be explained in the same way as the positive correlation between litter size at birth and plasma progesterone concentration at farrowing. Namely, sows giving birth to larger litters will have greater feto-placental production of progesterone. Hence plasma progesterone will decline more slowly in sows giving birth to larger litters.

Despite variance in the disappearance rate constant resulting from different sow fatness and different litter sizes, there was no correlation between the disappearance rate constant and the onset of lactogenesis as determined by litter weight gain in the first 2 d of life. De Passillé *et al.* (1993) observed a positive correlation between plasma progesterone concentration in sows in the 48 h following farrowing and in 3 d weight gains of piglets. The only correlation between progesterone and piglet weight gain in this experiment was that between the decline in plasma progesterone concentration in the 6 h following farrowing and piglet weight gain to 6 d of age. This does suggest that the more rapidly progesterone declines immediately after farrowing, the better the establishment of lactation. However this effect ceased to be important as the piglets grew to heavier weights.

One unexpected observation was the positive correlation between plasma progesterone concentration in late gestation and litter weight at birth. This effect was over and above that of sow liveweight and probably reflects feto-placental progesterone production in late gestation (Craig, 1982). A larger litter would have a greater feto-placental mass and therefore greater potential progesterone production.

It is interesting at this point to compare the results of Chapter 2 with those of the current chapter regarding apparent progesterone metabolism. In Chapter 2, peripubertal ovariectomised gilts were used as a model for sows in late gestation and during farrowing to establish relationships between feed intake and body fatness with metabolic clearance of progesterone. To recap, it was found that doubling feed intake increased MCR of progesterone, resulting in lower plasma progesterone concentrations and shorter progesterone half-life. Fatness had no effect on either parameter. However, in the present experiment using farrowing sows, exactly the opposite observations were made. Namely, feed intake had no effect on plasma progesterone concentrations but the disappearance rate constants were smaller in fatter sows resulting in a longer half-life in these animals. It therefore seems that progesterone-infused peripubertal ovariectomised gilts are not a perfect model for studying the progesterone kinetics of sows in late gestation through to early lactation.

3.4.6 Lactogenesis and piglet performance

There was no difference between the two treatments in the liveweight gain of piglets in the first two days of life suggesting that time of onset of lactogenesis was similar for both treatments. There was also no difference between treatments in the incidence of agalactia. Interestingly, of the nine cases in which mean piglet growth was less than or equal to zero in the first two days of life, three of these cases occurred in the same sow i.e. in each of her three parities (two on high and one on normal treatment).

Regardless of treatment, piglets grew similarly from birth through to weaning. This is in agreement with the findings of Hillyer and Phillips (1980), Pond *et al.* (1981) and Sterling and Cline (1986). Therefore, it seems that the metabolic state of the sow at farrowing is not critically important in determining her ability to establish and maintain lactation. All sows became catabolic during lactation but their ability to adapt to this situation was not affected by their metabolic state at farrowing. In contrast Cromwell *et al.* (1989) recorded an increase in growth rate of 130 g per piglet over 21 d (6.2 g/d) for sows which had received extra feed in late gestation, but again this was observed only in sows which had been on the treatment for two or more gestation-lactation cycles and may have resulted from comparatively larger sow size.

In the current experiment, piglet weight gains in the first week of life were positively correlated to piglet birthweight, sow fatness and sow parity but were not related to sow feed intake (Equation 3.11). This is in agreement with many authors who have reported no relationship between piglet growth rate and sow feed intake in early lactation (King and Dunkin, 1985; Yang *et al.*, 1989; Pluske *et al.*, 1995). By 20 d of age, however, piglet growth rate was correlated to sow feed intake (Equation 3.14) although the effect of feed intake was small and explained less than 1 % of the variance in piglet growth rate.

3.4.7 Subsequent reproductive performance

Subsequent reproductive performance was not directly affected by feeding level in late gestation, but it was positively correlated to liveweight gain over the whole of gestation (Equation 3.15). This suggests that the pre-farrowing status of the sow did affect her fertility in the following reproductive cycle but that this effect was small (0.04 extra pigs per kg gain in gestation) compared to the other predictors of subsequent litter size. It also supports the idea that no single stage of one reproductive cycle should be considered in isolation since it is the cumulative effects of successive cycles which will determine the lifetime breeding performance of the sow (Cole, 1990). The current results suggest that the metabolic state of the sow in early lactation may affect the quality of follicles ovulating after weaning. It now appears that the status of the sow during gestation has some importance. However, in the current experiment, in which sows were weaned after a 25 d lactation, the metabolic state of the sow immediately prior to farrowing did not appear to affect subsequent reproductive performance. If the metabolic status of the sow is important 3 weeks prior to weaning, as the work of Koketsu (1994) and Tokach (1991) imply, then it may be that metabolic status immediately prior to farrowing will be more important in early weaned sows, i.e. weaned at or before 14 d after farrowing.

The data in this experiment suggest that feed intake in the last week or so prior to weaning is important in determining subsequent reproductive performance. Feed intake in the third week of lactation was a significant predictor of subsequent litter size whilst feed intakes in the first two weeks of lactation were not correlated to ensuing reproductive performance. This finding supports the work of Zak *et al.* (reported by Aherne *et al.*, 1995). Their experiment used primiparous sows with a standardised litter size of six piglets to investigate the effects of three patterns of feeding during lactation on subsequent reproductive performance. Sows were fed 1) to appetite for one hour three times per d throughout lactation, or 2) to appetite from farrowing until d 21 of lactation and then restricted to 50% of the to appetite intake for the remainder of lactation, or 3) sows were restricted to 50% of to appetite intake from farrowing to d 21 and then fed to appetite until d 28 of lactation. Pattern of feeding in lactation did not affect the pattern of LH secretion 12 h post-weaning, but both restricted groups had a longer WEI and lower ovulation rate than unrestricted sows. In addition there was a reduction in embryo survival in sows which were restricted during the last week of lactation so that this group had the lowest fertility of all the treatments. This experiment showed that the detrimental effects of low feed intake for three out of four weeks of lactation could be partially offset by adequate feeding in the week before weaning. In contrast, restricted feeding for just one week immediately prior to weaning, even after adequate feeding for the whole of the rest of lactation, was even more detrimental to subsequent reproductive performance.

3.5 Conclusions

Increasing feed intake in late gestation had little effect on sow performance. Sows which received higher feeding levels lost less backfat between d 98 of gestation and weaning and therefore should have greater longevity in the breeding herd. In this experiment increasing feed intake prior to farrowing did not cause increased incidence of agalactia, nor did it increase piglet birthweight, growth rates or mortality. High feed intake in the last two weeks of gestation did not affect lactation feed intake nor increase diet digestibility. Since sow feed intake in the period three to four weeks prior to weaning is thought to be important for subsequent reproductive performance, high intake feeding in late gestation may prove to be more important in early weaned sows.

Increased feed intake in late gestation did not change progesterone concentrations in sows nor increase the rate of decline of progesterone at farrowing. There was no difference in time of onset of lactogenesis between H and N sows as determined by piglet growth rates in the first two days of life. There was also no correlation between sow plasma progesterone concentration and time of onset of lactogenesis.

3.6 References

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Table 3.1 Distribution of sows from different parities to high or normal feed intake levels in late gestation

Feed intake level	**Parity 1**	**Parity 2**	**Parity 3**	**Total**
Normal	27	19	17	**63**
High	20	21	13	**54**
Total	**47**	**40**	**30**	**117**

Table 3.2 Composition of gestation and lactation diets

Ingredients	**Pregnant Sow**	**Lactating Sow**
Wheat	30.00	30.00
Barley	56.30	40.25
Tallow	2.00	3.00
Soybean meal (44 % CP)	7.00	12.00
Bran	0.00	10.00
Salt	0.50	0.50
Dicalcium phosphate	1.70	1.70
Limestone	1.40	1.40
Mineral/vitamin premix	1.00	1.00
HCl-Lysine	0.10	0.15
Nutrient composition		
DE (MJ/kg)	12.50	13.70
% Crude protein	13.70	15.40
% Lysine	0.56	0.74
% Calcium	0.90	0.90
% Phosphorus	0.70	0.70

[1] Supplied the following per kg of diet: Zinc, 120mg; Manganese, 12 mg; Iron, 150 mg; Copper, 12 mg; Selenium, 0.1 mg; Vitamin A, 5000 IU; Vitamin D_3, 500 IU; Vitamin E, 22 IU; Riboflavin, 12 mg; Niacin, 45 mg; Calcium Pantothenate, 24 mg; Choline Chloride, 840 mg; Vitamin B_{12}, 30 μg; Biotin, 200 μg.

Table 3.3 Average daily feed intakes of high and normal sows in late gestation and lactation

Feed Intake (kg/d)	**High**	**Normal**	**Significance**
From d 100 gestation until farrowing	3.93 (0.037)	2.33 (0.034)	$P < 0.001$
D 1 of lactation	3.30 (0.253)	4.00 (0.238)	N.S.
First week of lactation	4.98 (0.168)	5.13 (0.158)	N.S.
Second week of lactation	6.55 (0.205)	6.67 (0.191)	N.S.
Third week of lactation	7.65 (0.207)	7.31 (0.197)	N.S.
Overall lactation	6.61 (0.164)	6.63 (0.161)	N.S.

Table 3.4 Average daily feed intakes of sows from parities 1, 2 and 3 in late gestation and lactation

Feed Intake (kg/d)	**Parity 1**	**Parity 2**	**Parity 3**	**Significance**
D 100 gestation until farrowing	2.73^{a} (0.039)	3.12^{b} (0.042)	3.55^{c} (0.049)	$P<0.002$
D 1 of lactation	3.18^{a} (0.268)	4.08^{b} (0.296)	3.71^{ab} (0.335)	$P<0.05$
Week 1 of lactation	4.16^{a} (0.181)	5.41^{b} (0.195)	5.59^{b} (0.221)	$P<0.001$
Week 2 of lactation	5.54^{a} (0.221)	7.02^{b} (0.240)	7.28^{b} (0.265)	$P<0.001$
Week 3 of lactation	6.46^{a} (0.225)	7.85^{b} (0.253)	8.14^{b} (0.262)	$P<0.001$
Overall lactation	5.63^{a} (0.174)	7.00^{b} (0.199)	7.24^{b} (0.222)	$P<0.001$

Figures with different superscripts within rows are significantly different.

Table 3.5 Changes in liveweight and backfat thickness during late gestation and lactation in high and normal sows

	High	**Normal**	**Significance**
D 98 liveweight (kg)	202.9 (2.44)	208.7 (2.21)	N.S.
D 98 P2 backfat (mm)	19.2 (0.62)	19.5 (0.57)	N.S.
LWG breeding to d 98 (kg)	53.4 (2.12)	55.0 (1.96)	N.S.
Farrowing liveweight (kg)	207.8 (2.96)	205.2 (2.75)	N.S.
Farrowing P2 (mm)	19.5 (0.63)	18.0 (0.58)	N.S.
LWG breeding to farrowing (kg)	57.6 (2.47)	51.2 (2.30)	($P<0.058$)
LWG d98 to farrowing (kg)	3.9 (1.44)	-3.9 (1.39)	$P<0.001$
P2 gain backfat d98 to farrowing (mm)	0.2 (0.25)	-1.6 (0.23)	$P<0.001$
LWG farrow to weaning (kg)	-8.5 (2.17)	-3.1 (2.05)	N.S. ($P<0.075$)
P2 gain farrow to weaning (mm)	-2.7 (0.29)	-2.2 (0.27)	N.S.
LWG d98 to weaning (kg)	-4.2 (2.55)	-7.4 (2.38)	N.S.
P2 gain d98 to weaning (mm)	-2.6 (0.39)	-3.8 (0.36)	$P<0.05$

LWG = Liveweight gain
P2 gain = Gain in P2 backfat thickness

Table 3.6 Changes in liveweight and backfat thickness of sows in parities one, two and three during late gestation and lactation.

	Parity 1	**Parity 2**	**Parity 3**	**Significance**
D 98 liveweight (kg)	172.4^{a} (2.50)	206.3^{b} (2.72)	240.2^{c} (3.08)	$P<0.001$
D 98 P2 backfat (mm)	17.5^{a} (0.65)	18.4^{a} (0.70)	22.1^{b} (0.82)	$P<0.001$
LWG breeding to d 98 (kg)	59.6 (2.25)	52.4 (2.45)	50.6 (2.77)	$P<0.05$
Farrowing liveweight (kg)	172.5^{a} (3.14)	207.1^{b} (3.48)	240.0^{c} (3.84)	$P<0.001$
Farrowing P2 backfat (mm)	17.5^{a} (0.66)	17.3^{a} (0.72)	21.5^{b} (0.83)	$P<0.001$
LWG breeding to farrowing (kg)	59.5 (2.62)	53.3 (2.91)	50.4 (3.21)	N.S. ($P<0.07$)
LWG d98 to farrowing (kg)	0.24 (1.590)	-0.04 (1.764)	-0.22 (1.946)	N.S.
P2 gain d98 to farrowing (mm)	-0.03^{a} (0.26)	-1.19^{b} (0.28)	-0.98^{b} (0.33)	$P<0.01$
LWG farrow to weaning (kg)	-7.36 (2.302)	-1.71 (2.550)	-8.37 (2.866)	N.S.
P2 gain farrow to weaning (mm)	-2.74 (0.320)	-2.59 (0.335)	-1.89 (0.377)	N.S.
LWG d98 to weaning (kg)	-8.14 (2.697)	-0.67 (2.934)	-8.72 (3.390)	N.S.
P2 gain d98 to weaning (mm)	-3.00 (0.422)	-3.79 (0.448)	-2.89 (0.498)	N.S.

Figures with different superscripts within rows are significantly different.

Table 3.7 Plasma progesterone concentrations during late gestation and early lactation in sows fed high or normal intakes in late gestation

	High	Normal	Significance
Plasma P4 (ng/mL)			
Pre-treatment (d98 of gestation)	24.4 (1.25)	25.8 (0.98)	N.S.
During treatment (d 109) - preprandial*	26.1 (0.95)	25.1 (0.81)	N.S.
During treatment (d 109) -postprandial*	25.7 (0.88)	24.8 (0.71)	N.S.
At farrowing*	8.3 (0.70)	8.4 (0.57)	N.S.
6 h post-farrowing	5.9 (0.56)	6.2 (0.45)	N.S.
Disappearance rate constant	0.019 (0.003)	0.025 (0.002)	N.S.
Half life (h)	42.3 (5.32)	31.4 (4.44)	N.S.

* Pre-treatment value used as covariate

Table 3.8 Plasma progesterone concentrations during late gestation and early lactation in sows of parities one, two and three

	Parity 1	**Parity 2**	**Parity 3**	**Significance**
Plasma P4 (ng/mL)				
Pre-treatment (d98 of gestation)	21.9^{a} (1.42)	25.7^{b} (1.12)	27.7^{b} (1.57)	P<0.05
During treatment (d 109) - preprandial*	24.4 (1.16)	25.8 (0.89)	26.5 (1.26)	N.S.
During treatment (d 109) -postprandial*	24.8 (1.07)	24.9 (0.81)	25.1 (1.13)	N.S.
At farrowing*	8.4 (0.88)	7.8 (0.65)	9.0 (0.93)	N.S.
6 h post-farrowing	5.8 (0.67)	5.3 (0.53)	7.0 (0.72)	N.S.
Disappearance rate constant	0.025 (±0.003)	0.022 (±0.003)	0.020 (±0.003)	N.S.
Half life (h)	30.5 (±6.65)	39.3 (±5.12)	40.7 (±6.57)	N.S.

Figures with different superscripts within rows are significantly different.
* Pre-treatment value used as covariate

Table 3.9 Piglet performance data for sows fed either high or normal feeding levels in late gestation

	High	**Normal**	**Significance**
Total born	11.2 (0.40)	11.1 (0.37)	N.S.
Stillborn	0.8 (0.18)	0.6 (0.16)	N.S.
Weight of litter at birth (kg)	16.5 (0.56)	16.4 (0.51)	N.S.
Birthweight of liveborn piglets (kg)	1.53 (0.035)	1.52 (0.032)	N.S.
Weight gain in first 2 d of life (kg)	0.22 (0.028)	0.23 (0.026)	N.S.
Litter size after 48h*	9.6 (±0.27)	9.3 (±0.25)	N.S.
Liveweight gain to 7 d of age (kg)	1.16 (0.060)	1.15 (0.055)	N.S.
Daily piglet liveweight gain to 20 d (kg/d)	0.243 (0.008)	0.244 (0.008)	N.S.
Number of pigs weaned	8.6 (0.298)	8.4 (0.266)	N.S.
Total weight of pigs weaned (kg)	67.4 (2.394)	66.7 (2.116)	N.S.
Mean piglet weight at weaning (kg)	7.9 (0.19)	8.0 (0.16)	N.S.
Daily piglet liveweight gain to weaning (kg/d)	0.256 (0.006)	0.257 (0.005)	N.S.
Mortality	13.7 (2.10)	17.0 (1.93)	N.S.

* Littersize at 48 h after cross-fostering to balance sizes across treatments.

Table 3.10 Piglet performance data for sows of parities one, two and three.

	Parity 1	**Parity 2**	**Parity 3**	**Significance**
Total born	10.1^{a} (0.43)	11.4^{ab} (0.46)	11.9^{b} (0.52)	$P<0.05$
Stillborn	0.4^{a} (0.19)	0.5^{a} (0.20)	1.2^{b} (0.23)	$P<0.05$
Weight of litter at birth (kg)	14.0^{a} (0.60)	17.6^{b} (0.66)	17.8^{b} (0.71)	$P<0.001$
Birthweight of liveborn piglets (kg)	1.40^{a} (0.04)	1.62^{b} (0.04)	1.56^{b} (0.05)	$P<0.001$
Weight gain in first 2 d of life (kg)	0.22 (0.29)	0.23 (0.32)	0.23 (0.37)	N.S.
Litter size after 48h*	9.2 (0.28)	9.6 (0.30)	9.5 (0.36)	N.S.
Liveweight gain to 7 d of age (kg)	0.98^{a} (0.06)	1.24^{b} (0.07)	1.24^{b} (0.08)	$P<0.01$
Daily piglet liveweight gain to 20 d (kg/d)	0.214^{a} (0.009)	0.249^{b} (0.009)	0.267^{b} (0.011)	$P<0.001$
Number of pigs weaned	8.1 (0.31)	9.0 (0.32)	8.4 (0.40)	N.S.
Total weight of pigs weaned (kg)	56.2^{a} (2.48)	74.4^{b} (2.58)	70.6^{b} (3.19)	$P<0.001$
Mean piglet weight at weaning (kg)	6.9^{a} (0.19)	8.4^{b} (0.20)	8.5^{b} (0.25)	$P<0.001$
Daily piglet liveweight gain to weaning (kg/d)	0.228^{a} (0.006)	0.266^{b} (0.006)	0.274^{b} (0.008)	$P<0.001$
Mortality	15.8 (2.23)	11.0 (2.43)	19.3 (2.73)	N.S.

Figures with different superscripts within rows are significantly different.
* Littersize at 48 h after cross-fostering to balance sizes across treatments.

Table 3.11 Diet digestibility in early lactation for sows fed high or low feed intakes in late gestation

Digestibility %	High	Normal	Significance
Dry matter			
Pre-treatment (late gestation)	78.9 (0.48)	78.7 (0.42)	N.S.
Days 2-4 of lactation	78.4 (0.76)	77.3 (0.67)	N.S.
Days 5-7 of lactation	77.8 (0.66)	76.5 (0.58)	N.S.
Crude protein			
Pre-treatment (late gestation)	77.2 (0.73)	77.6 (0.64)	N.S.
Days 2-4 of lactation	81.2 (0.88)	79.3 (0.80)	N.S.
Days 5-7 of lactation	80.6 (0.86)	78.5 (0.78)	N.S.
Energy			
Pre-treatment (late gestation)	79.3 (0.52)	79.0 (0.46)	N.S.
Days 2-4 of lactation	78.5 (0.83)	77.6 (0.73)	N.S.
Days 5-7 of lactation	78.0 (0.72)	76.9 (0.64)	N.S.

Table 3.12 Subsequent reproductive performance of sows fed either high or normal intakes in late gestation

	High	**Normal**	**Significance**
Weaning to estrus interval (days)	5.3 (0.94)	6.9 (0.88)	N.S.
Subsequent litter size	12.2 (0.51)	12.4 (0.45)	N.S.

Table 3.13 Subsequent reproductive performance of sows of parities one, two and three

	Parity 1	**Parity 2**	**Parity 3**	**Significance**
Weaning to estrus interval (days)	6.8 (0.86)	5.9 (1.09)	5.6 (1.34)	N.S.
Subsequent litter size	11.1 (0.46)	12.8 (0.52)	12.9 (0.75)	$P<0.05$

Figure 3.1 Blood sampling times to sample for plasma progesterone

Ear vein blood samples were taken from 44 sows at these times

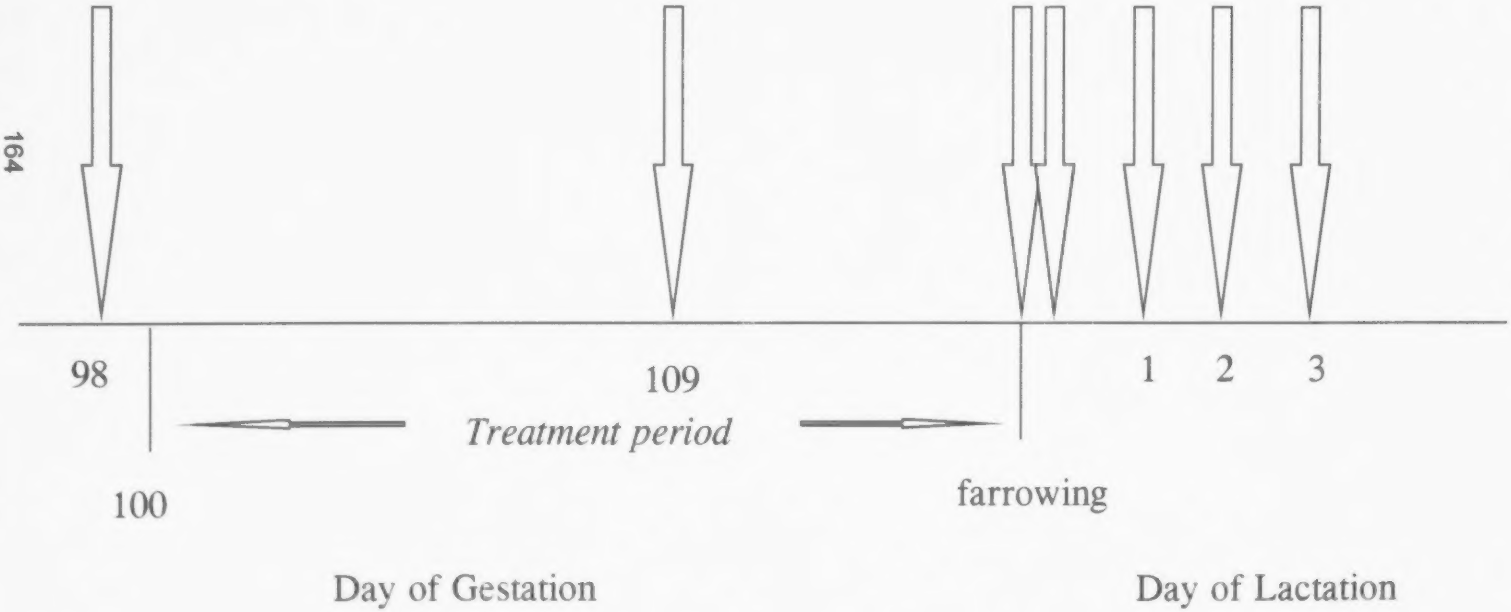

98
100
109
Treatment period
farrowing
1
2
3
Day of Gestation
Day of Lactation

Figure 3.2 Lactation feed intake of sows fed high or low intakes in late gestation

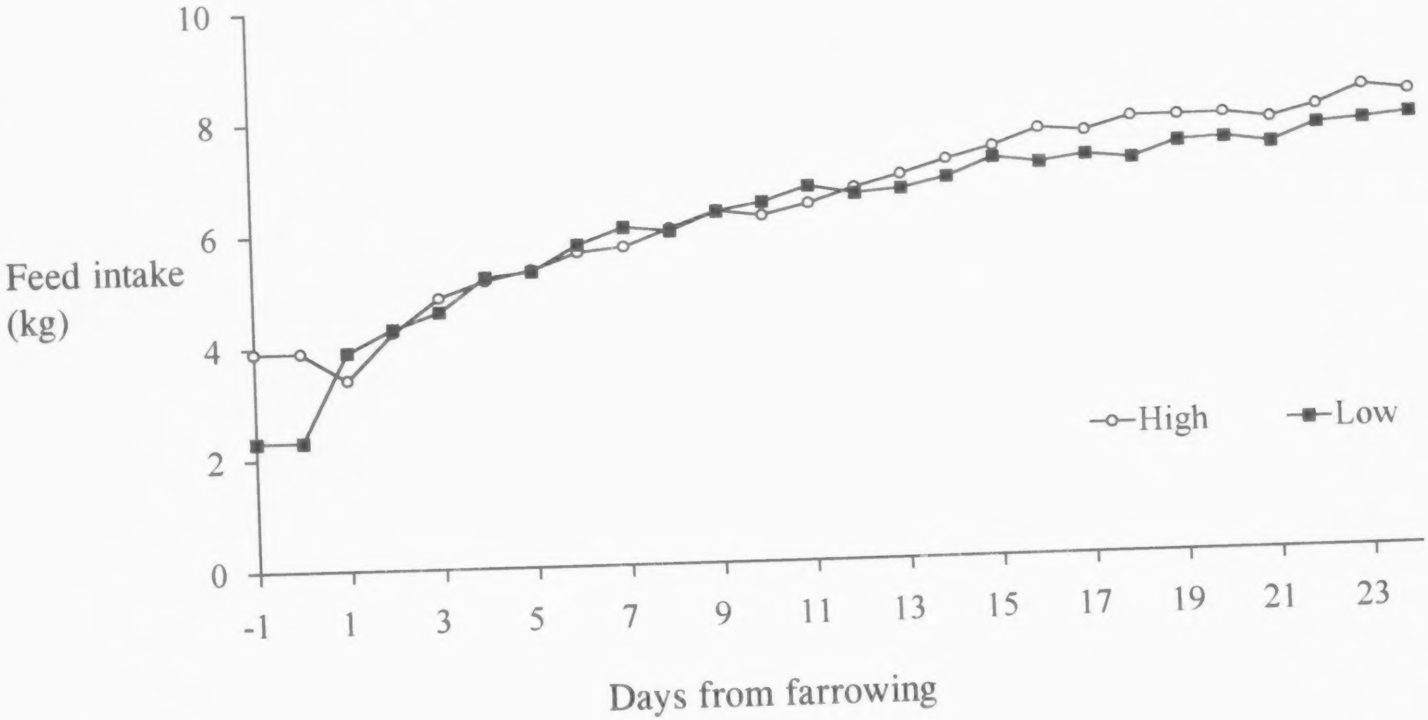


Figure 3.3 Distribution of mean lactation feed intakes for sows of parities 1, 2 and 3

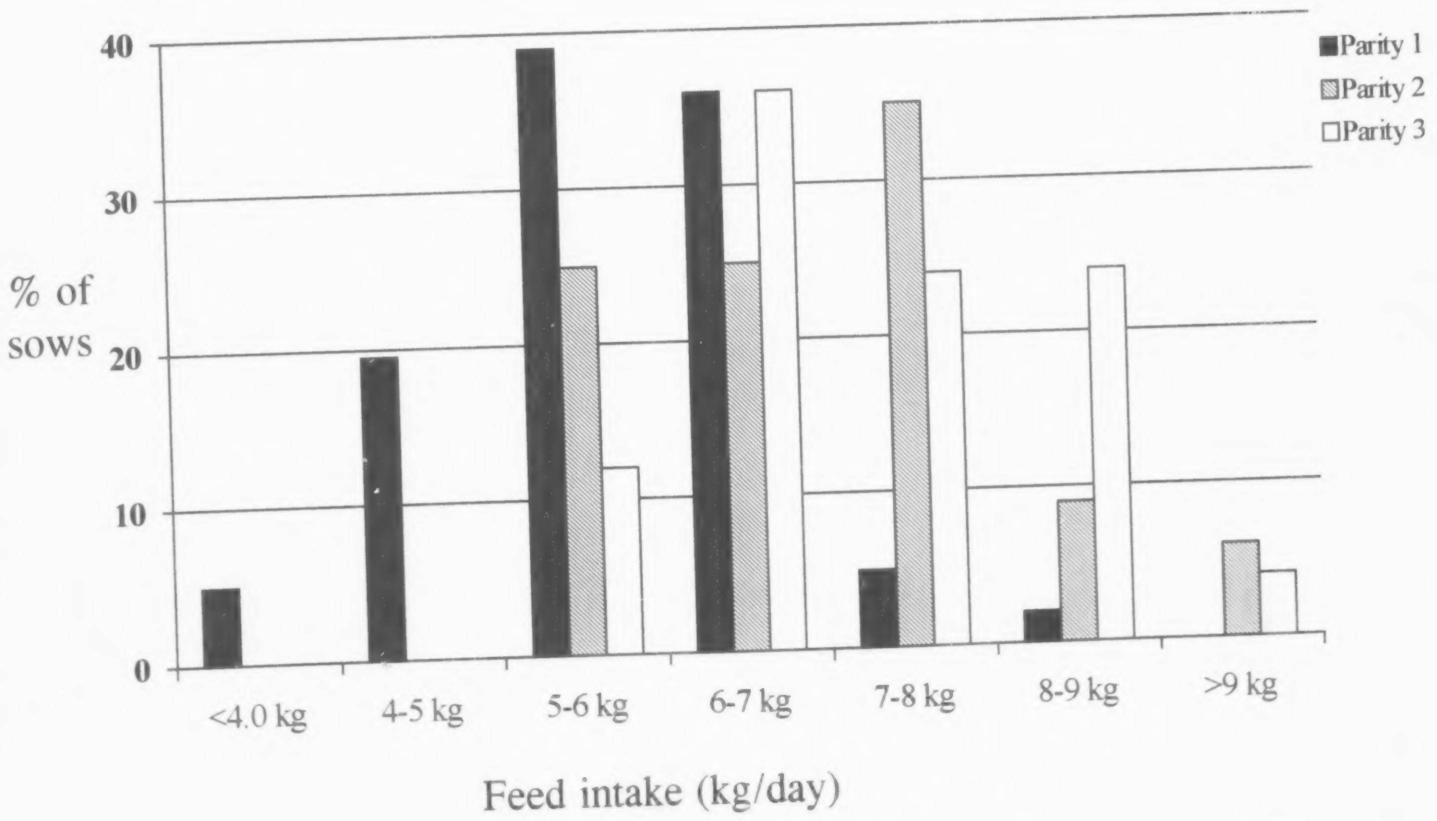
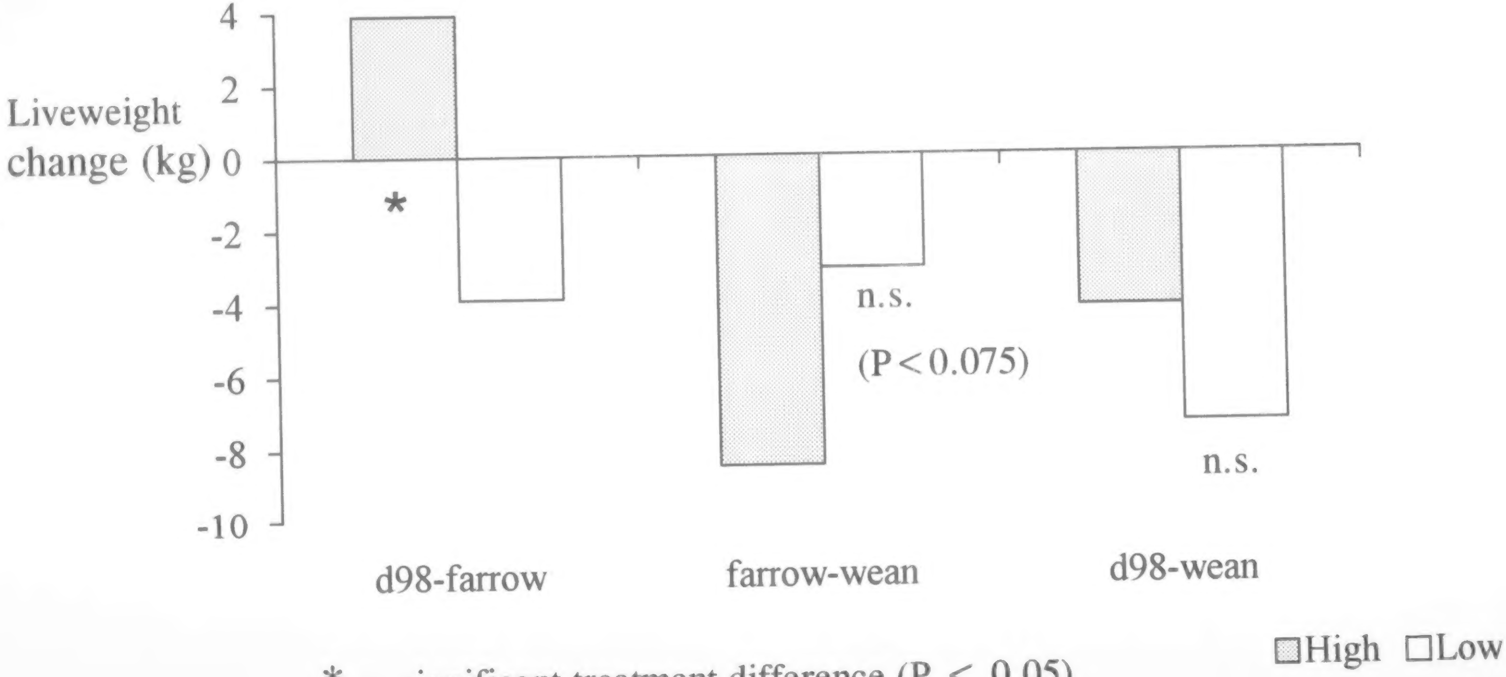
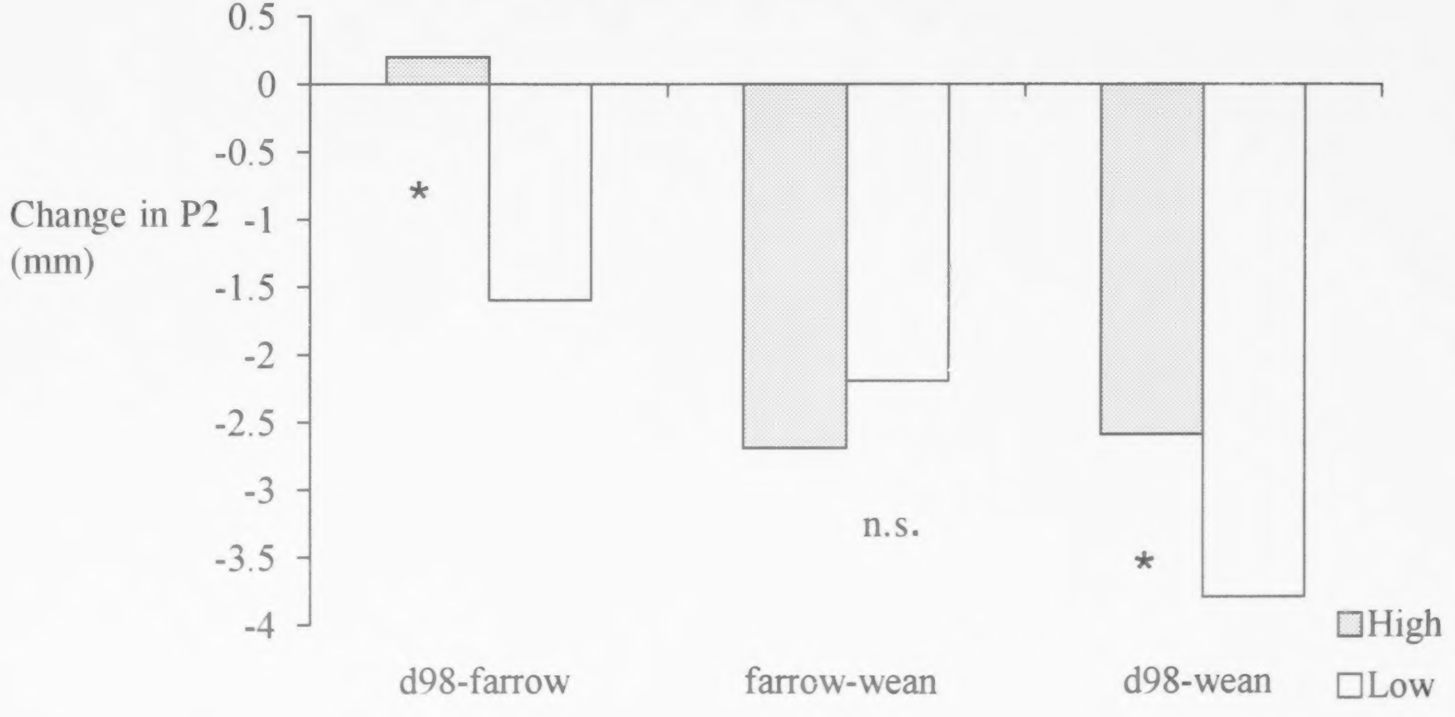


Figure 3.4 Liveweight changes in sows fed high or low intakes in late gestation



* = significant treatment difference (P < 0.05)

Figure 3.5 P2 backfat changes in sows fed high or low intakes in late gestation



* = significant treatment difference (P < 0.05)

Figure 3.6 Distribution of P2 backfat at farrowing for sows of parities 1, 2 and 3

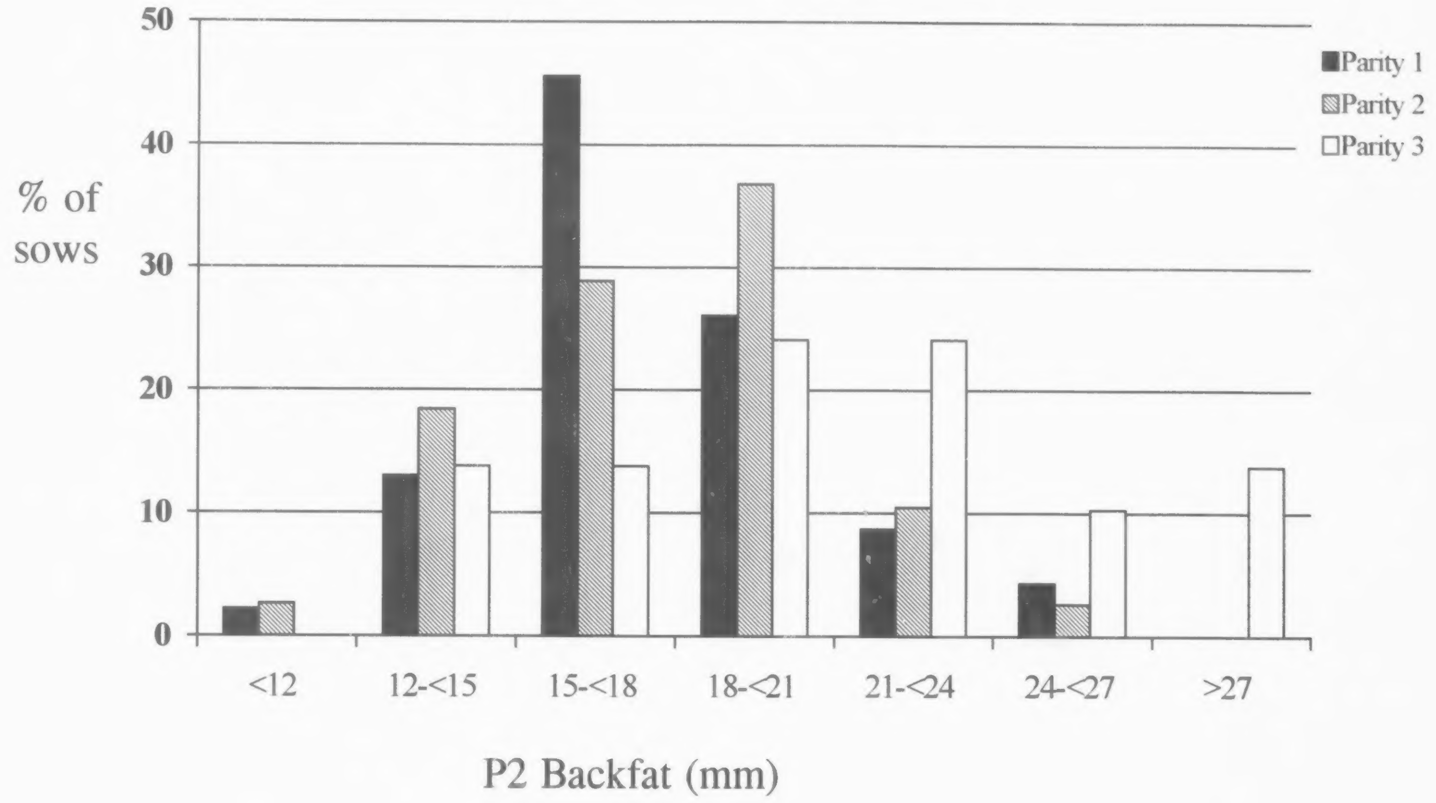


Figure 3.7 Distribution of P2 backfat loss between farrowing and weaning for sows of parities 1, 2 and 3

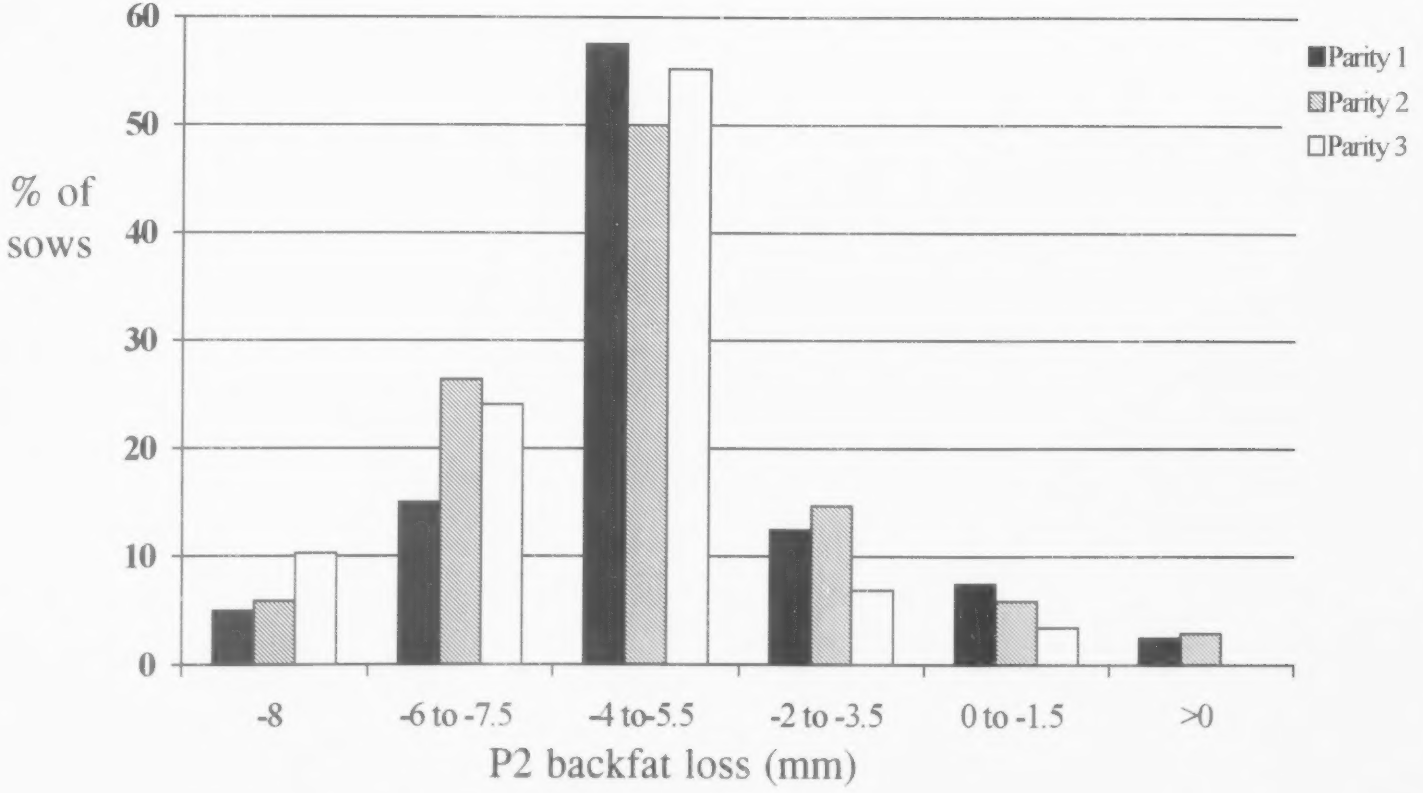
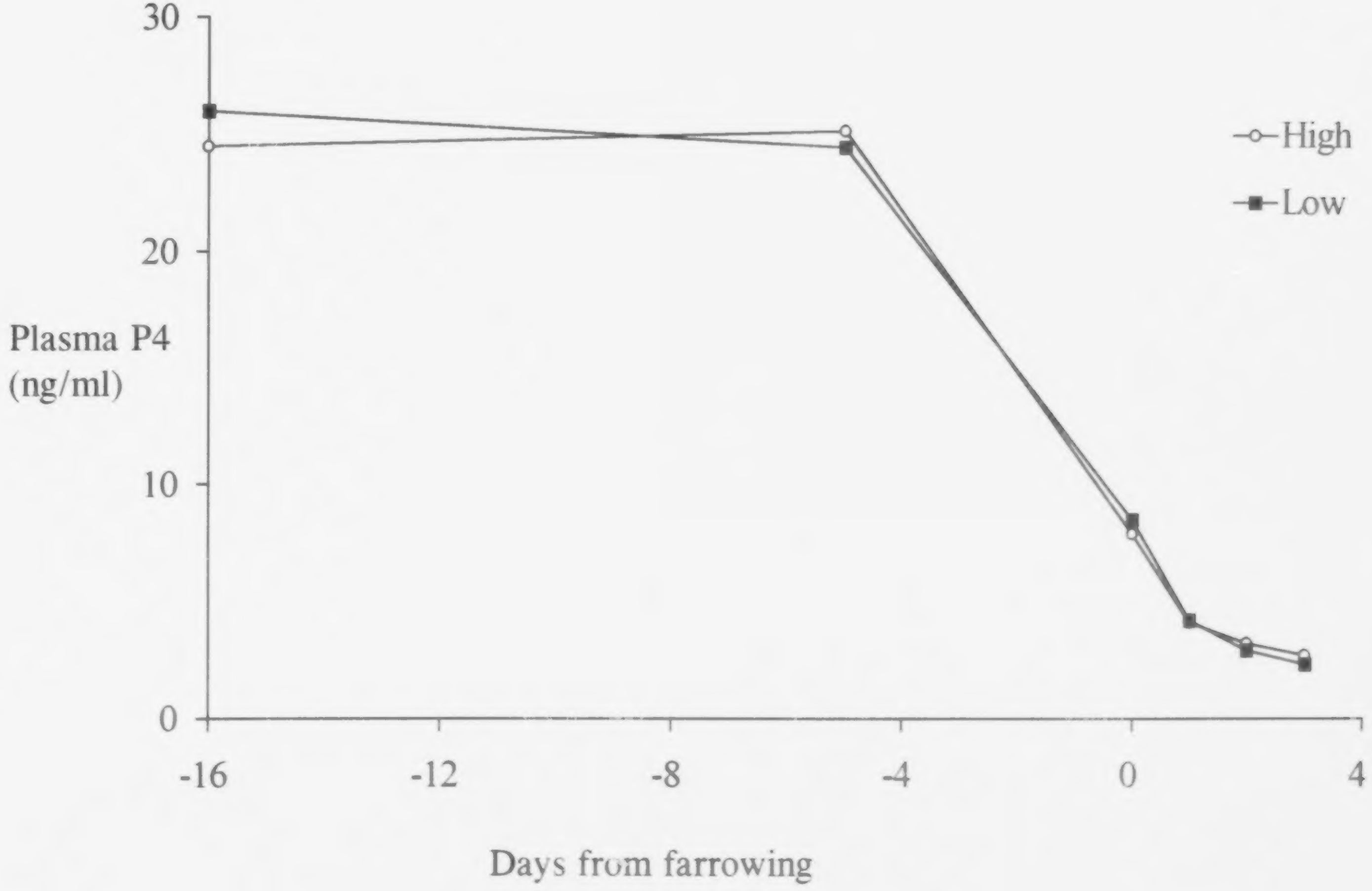


Figure 3.8 Plasma progesterone in sows fed high or low intakes in late gestation



CHAPTER 4

Metabolic Status of Sows during Early Lactation Affects Performance and Reproductive Potential

4.1 Introduction

Lactogenesis II is the transition from colostrum production to copious milk production which occurs at the time of farrowing in the sow (Hartmann and Holmes, 1989) and is triggered by the periparturient decline in plasma progesterone concentration (Martin *et al.*, 1978; Gooneratne *et al.*, 1979; Willcox *et al.*, 1983). The rate of progesterone decline may affect the efficacy with which a successful lactation is achieved (Liptrap, 1980). De Passillé *et al.* (1993) found that there was a negative correlation between sow plasma progesterone levels during the 48 hours after farrowing and piglet growth rates in the first three d of life. Progesterone decline in the first 6 h postpartum was negatively correlated to piglet growth rate in the first week of life (see Chapter 3). These findings suggest that the rate of decline of progesterone after farrowing may be crucial to the satisfactory establishment of early lactation. In Chapter 2 increasing feed intake of ovariectomised, progesterone-infused gilts increased clearance rate of progesterone resulting in reduced plasma progesterone concentration. However, in sows, manipulating feed intake level during late gestation did not affect plasma progesterone concentration or change rate of plasma progesterone decline after parturition (Chapter 3). It is possible that feed intake in the first few days of lactation may affect rate of plasma progesterone decline after parturition.

In addition to progesterone withdrawal, the sow also requires a periparturient peak of prolactin to establish lactation successfully (Whitacre and Threlfall, 1981). Taverne *et al.* (1982) clearly demonstrated that lactation was completely prevented when prolactin secretion in the periparturient period was inhibited by feeding 10 mg bromocriptine twice daily from d 111 of gestation to d 1 postpartum. Although the absence of prolactin inhibits lactation, it is not known whether the height of the periparturient peak affects the success of the subsequent lactation, as does the failure of progesterone to decline rapidly. Dusza (cited by Boyd *et al.* 1995) injected sows with prolactin on d 1 of lactation and obtained an 8% increase in piglet growth rate. However other attempts to stimulate lactation by increasing sow prolactin levels in the periparturient period have proved unsuccessful (Crenshaw *et al.* 1989; King *et al.*, 1996), perhaps because of inappropriate timing of prolactin administration. Akers *et al.* (1981) have shown in cattle that the time window for the prolactin peak is quite narrow.

Prolactin and insulin are the main galactopoietic hormones in the sow (Collier *et al.*, 1984; Forsyth, 1986; Vernon, 1989) and Pettigrew *et al.*(1993) have suggested that IGF-1 concentrations may also determine milk yield. Plasma levels of these hormones are increased by increased feed intake (Rojkittikhun *et al.*, 1993; Weldon *et al.*, 1994a, Zak, personal communication), however, many experiments have shown that sows receiving different lactation feed intakes (e.g. restricted versus *ad libitum*) showed no difference in milk production during the first two to three weeks of lactation (Lythgoe, 1987; Yang *et al.* 1989; Prunier *et al.*, 1993). It is clear that the relationship between circulating hormone levels and milk production is not straight forward. It is possible that the increased hormone levels regulate the degree of mobilisation of body reserves rather than promoting milk production *per se* and that these three hormones need only be present in a permissive capacity in early lactation.

Segregated early weaning is becoming a popular management strategy in North America because of the health benefits accruing to the weaned piglets (Wiseman *et al.*, 1994). However, early weaning of sows has been associated with rebreeding problems such as longer WEI (Svajgr *et al.*, 1974; Cole *et al.* 1975; Varley and Cole, 1976; 1978; Xue *et al.*, 1993) and smaller subsequent litter size (Clark and Leman, 1987; Xue *et al.*, 1993). Svajgr *et al.* (1974) and Varley and Cole (1978) observed that ovulation rate was not affected by length of lactation but shorter lactations resulted in increased embryo mortality. This may be due to incomplete uterine involution and therefore failure to establish optimal uterine conditions for the implanting embryos, however it may equally be due to a poorer quality of embryo resulting from inadequate follicular development prior to ovulation (Foxcroft *et al.*, 1995). It is well established that low feed intake in lactation is associated with reduced subsequent reproductive performance following conventional length lactations, especially in primiparous sows (King and Williams, 1984; Hughes, 1993; Yang *et al.*, 1989; Baidoo *et al.*, 1992).

Recently workers from the University of Minnesota have suggested that feed restriction in the first two weeks of lactation can affect the fertility of the sow after weaning (Tokach *et al.*, 1992b; Koketsu, 1994). In this experiment the effect of restricted feed intake during lactation on follicular development and LH pulsatility was measured on d 14 of lactation. Follicular development and LH pulsatility can be used as measures of reproductive function.

4.1.1 Hypotheses

Based on the literature discussed it was hypothesised that;

- Plasma progesterone concentrations will decline more rapidly postfarrowing in sows with a higher feed intake.
- Progesterone and prolactin concentrations in plasma during the periparturient period will both determine sow potential lactation performance.
- There will be no difference in piglet growth rate between *ad libitum* and restricted sows. *Ad libitum* fed sows will have higher plasma prolactin, IGF-1 and insulin concentrations and will lose less weight than restricted sows.
- LH pulsatility and ovarian follicular development will be suppressed in restricted sows compared to *ad libitum* fed sows.

Primiparous sows were used in this study because the problems of reduced feed intake and high tissue loss in lactation with corresponding reduced reproductive performance are most evident in primiparous sows (reviewed by Cole, 1990).

4.2 Materials and Methods

Sixteen primiparous Camborough sows were used in this experiment. On d 109 of gestation the sows were moved into individual farrowing crates in a farrowing room. Farrowing stalls were totally slatted and had covered side creep areas containing heat lamps and overlays. Room temperature was maintained at 18°C ± 2°C. All sows received the lactation diet containing 14.4 MJ DE/kg, 20.5 % crude protein and 1.11 % lysine (Table 4.1) from d 109 of gestation until weaning. Sows were surgically fitted with a silastic catheter (Dow Corning Corporation, Michigan, USA) into the right jugular vein four to nine days before farrowing. The animals were anaesthetised with 'Intraval' (5% solution of 5-ethyl-5-(1-methyl-butyl)-2-thiobarbituric acid, MTC Pharmaceuticals, Cambridge, Ontario, Canada; Dosage: 0.17 mL 'Intraval' per kg BW), administered via an ear vein whilst the sows were restrained with a nose snare. Anaesthesia was maintained with a closed-circuit system of halothane and oxygen. Catheters were exteriorised through the dorsal surface of the neck. Sows were randomly allocated to one of the following treatments;

- **AL sows** - allowed *ad libitum* access to feed throughout a 14 ± 0.2 d lactation.
- **R sows** - restrict fed throughout a 14 ± 0.2 d lactation. These sows received 1 x maintenance energy in week one of lactation followed by 1.5 x maintenance energy in week two of lactation. Maintenance requirement was assumed to be 460 kJ DE/kg $BW^{0.75}$ (NRC, 1988). Sows were weighed within 24 h of farrowing and on

d 3, 7, 10 and 13 of lactation. Daily feed allowance was based on the sow's most recent weight .

During lactation AL sows had food in front of them at all times with two weigh backs each day, before the morning and evening feedings. R sows were fed half their ration at 8 am and half at 4 pm. Feed intakes were recorded daily. Sows had access to water at all times. Litter size was standardised across treatments within 48 h of parturition. On the day of birth, piglets received iron supplementation, their teeth and tails were clipped and their ears were notched. Male piglets were castrated between 7 and 9 d of age. Sows and their litters were weighed within 24 h of farrowing and then on d 3, 7, 10, and 13 of lactation, and at weaning. Backfat was measured on each of these d using an ultrasonic probe (Scanoprobe 11, Scanco, Ithaca, NY) at the P2 measurement site i.e. at the last rib and 65 mm from the midline. Milk samples were collected from at least 4 active teats on d 5 and 10 of lactation following injection of 4 IU of oxytocin. Approximately 5 mL of milk was removed from each sampled gland. Milk samples were frozen immediately at -30°C and stored at this temperature until analysis.

5 mL blood was sampled from all sows within 6 h of the start of farrowing and then every 6 h for 72 h. Blood samples were collected every 15 min from 7 am to 7 pm on the day before weaning and then hourly for a further 12 h. Blood was divided between heparinised and sodium fluorinated tubes. All blood samples were centrifuged within 1 h of collection at 1500 x *g* for 15 min at 4°C. Six hourly and hourly samples

were centrifuged within 10 min of collection. Plasma was collected and stored at -30°C until analysis for prolactin, insulin, IGF-1, LH, progesterone and glucose.

All sows were slaughtered at a commercial abattoir within 4 h of weaning. The ovaries were removed and follicles with diameter greater than or equal to 3 mm were counted and measured.

4.2.1 Hormone analyses

In all the hormone analyses, sows from both treatments were represented in every individual assay.

4.2.1.1 Progesterone

Only the plasma samples collected in the 72 hours after farrowing were analysed for progesterone using a Coat-A-Count Progesterone kit (Diagnostic Products Corporation, Los Angeles, USA) validated as a direct assay with porcine plasma. No significant deviation from parallelism was apparent from assaying 100, 50 and 25 µl of a standard plasma pool. Accuracy of the assay was determined by measuring known amounts of unlabelled hormone in a pool of porcine plasma, recovery was 102 ± 2.3%. Sensitivity of the assay, defined as 85% of total binding, was 0.02 ng/mL. The intra- and inter-assay coefficients of variation for the progesterone assays were 1.4% and 8.5%, respectively. Known amounts of unlabelled hormone in a pool of porcine plasma were run with each assay, cold recoveries averaged 105 ± 3.1%.

4.2.1.2 Prolactin

Plasma samples were assayed for prolactin in two assays using the homologous double antibody RIA described by de Passillé *et al.* (1993). No significant deviation from parallelism was evident from assaying 400 to 25 μl of a standard plasma pool. The mean intra- and inter-assay coefficients of variation were 8.6% and 5.4%, respectively. The mean assay sensitivity was 0.09 ng prolactin/tube, as defined by (mean Bmax -(2*mean Bmax sd)/mean Bmax)*100.

4.2.1.3 Insulin

Plasma samples were analysed for insulin in five assays using the double antibody RIA described by de Boer and Kennelly (1989) with modifications as described by de Passillé *et al.* (1993). No significant deviation from parallelism was evident from assaying 100 to 12.5 μl of a standard plasma pool. The assay sensitivity was 8 pg/tube, as defined by (mean Bmax -(2*mean Bmax sd)/mean Bmax)*100. The mean intra- and inter-assay coefficients of variation were 4.5% and 4.9%, respectively.

4.2.1.4 Insulin-like growth factor 1 (IGF-1)

Plasma IGF-1 concentrations were determined in four assays by double antibody radioimmunoassay after acid-ethanol extraction as described by Cosgrove *et al.* (1992). Extraction efficiency was routinely estimated to be greater than 95% and therefore no correction factor was applied to the data. No significant deviation from parallelism was evident from assaying 400 to 12.5 μl of a standard plasma pool. The assay sensitivity was 6 pg IGF-1/tube, as defined by (mean Bmax -(2*mean Bmax sd)/mean

Bmax)*100. The mean intra- and inter-assay coefficients of variation were 2.7% and 9.9%, respectively.

4.2.1.5 Luteinising hormone (LH)

Plasma LH concentrations were determined in duplicate 200 µl samples by the double antibody RIA described by Cosgrove *et al.* (1992), with the following modifications. Precipitation of the antibody-bound hormone was enhanced by diluting the locally raised second antibody, horse anti-goat gammaglobulin (HAGGG), 1:40 in 1% egg albumin/PBS buffer and pre-incubating with an equal volume of 6% (w/v) polyethylene glycol (PEG 8000: Carbowax) for 24 h. 400 µL of the HAGG-PEG solution was added to each assay tube and incubated for a minimum of 16 h at 4°C before centrifugation and aspiration. Reproducible standard curves were obtained using standard potencies of 2.4 to 5000 pg/tube of the LH preparation SDG-2-65 (0.96-1.18 x NIH oLH-s19). The LH hormone used for iodination was IVO-DHO-pLH, kindly supplied by the Research Institute for Animal Production, The Netherlands.

The mean assay sensitivity, defined as (mean Bmax -(2*mean Bmax sd)/mean Bmax)*100, was 0.01 ng/tube. No significant deviation from parallelism was evident from assaying 200, 100, 50 and 25 µl of a standard plasma pool. The mean intra- and inter-assay coefficients of variation for the two assays were 7.4% and 4.9%, respectively.

4.2.2 Glucose analysis

Plasma samples from sodium fluorinated tubes were pooled for analysis of glucose as follows;

- day 1 = four x six hourly samples taken in the first 24 h after farrowing.
- day 3 = four x six hourly samples taken between 48 - 72 h after farrowing.
- in lactation = 24 x hourly samples taken on the day before weaning.

The three pooled plasma samples for each sow were analysed for glucose using a hexokinase-reaction colorimetric kit (Sigma Chemical Co. Ltd., Poole, Dorset, England).

4.2.3 Analysis of milk composition

Milk samples were analysed for fat, protein and lactose using an infra red detector (Milko-scan 605, A/S N. Foss Electric, Denmark).

4.2.4 Statistical analyses

Data for sow feed intake, BW and fatness, and the changes in BW and fatness, hormone concentrations, glucose concentrations, piglet numbers and growth rates were analysed using analyses of variance. Sources of variation were replicate, feed intake (*ad libitum* or restricted) and the interaction between them. The error term was sow within replicate by feed intake. Differences between hormone concentrations after farrowing and at the end of week 2 of lactation were analysed as a repeated measure with sources of whole plot variance as for the model using feed intake and the repeated measure as five time periods (farrowing, d 1, d 2, d 3 and d 13 of lactation). Hormone concentrations on d 1, d 2 and d 3 were the mean of the 4 samples taken on that day, starting from the sample taken 6 h after farrowing on d 1. Although the experiment was designed to compare only two feed intake levels there was a wide range of feed intake between the 16 sows (see Figure 4.1) and therefore multiple regression analyses were performed to further clarify relationships between measured variables and feed intake. Multiple regression analyses were also performed between plasma hormone and glucose concentrations and piglet growth rate (used as an indication of milk yield). All statistical analyses were computed using Systat (Wilkinson, 1990).

LH pulse frequency for each sow was assessed by visual appraisal of LH profiles. A pulse was considered to have occured when LH concentration increased within two sampling intervals and then declined at a rate approximating the half-life of the hormone, with at least 3 sampling intervals between the defined peak and the succeeding baseline (Cosgrove *et al.*, 1991).

4.3 Results

4.3.1 Lactation length

The commercial abattoir used to slaughter the sows was only available on Mondays, Wednesdays and Fridays, therefore sows were killed after a minimum lactation length of 13 d up to a maximum of 15 d. As a result AL sows had a significantly longer mean lactation length than R sows, 14.5 versus 13.3 d ($P < 0.01$). Where appropriate data have been adjusted to a standard lactation length of 13 days.

4.3.2 Lactation feed intake

AL sows ate more than R sows throughout lactation with mean daily feed intakes of 4.4 versus 1.7 ± 0.29 kg/d ($P < 0.001$) in week 1 and 5.1 versus 2.3 ± 0.32 kg/d ($P < 0.001$) in week 2, respectively. The pattern of feed intake over the whole of lactation is shown in Figure 4.2. AL sows had a wide range of feed intakes varying from 2.7 to 5.6 kg/d in wk 1 and from 3.7 to 7.1 kg/d in wk 2.

4.3.3 Sow liveweight and backfat thickness

Sow liveweight and backfat data are presented in Table 4.2. There was no difference between the treatment groups in the liveweight or backfat thickness of sows at farrowing. Sows averaged 209 kg liveweight with a backfat thickness of 18.3 mm. R sows lost significantly more weight and backfat than AL sows in the first week of lactation (16.3 kg and 2.5 mm versus 2.5 kg and 1.3 mm, respectively, $P < 0.001$). AL sows actually gained an average of 1.2 kg BW during the first three days after farrowing whilst R sows lost 9.2 ± 2.10 kg over the same period. Both groups of sows lost similar amounts of liveweight and backfat in the second week of lactation. At weaning R sows were lighter than AL sows (178.3 versus 200.5 ± 5.92 kg respectively, $P < 0.05$), but were not significantly leaner.

Sow BW change in the first week of lactation was positively correlated to week 1 feed intake ($R^2 = 0.785$, $P < 0.001$), and likewise in the second week of lactation sow BW change and feed intake were correlated ($R^2 = 0.255$, $P < 0.05$). However, during the second week more of the variation in sow BW change was explained by also including litter weight gain in the regression, litter weight gain only became a significant factor in influencing BW change after feed intake had been included in the equation;

Equation 4.1
$$\mathbf{SBWC_{7\text{-}13} = \underset{(0.595)}{-\,0.242} - \underset{(0.000)}{0.001}\,LG_{7\text{-}13} + \underset{(0.090)}{0.377}\,FI_{7\text{-}13}}$$
$$(R^2 = 0.590,\ P < 0.003).$$

Where; $SBWC_{7\text{-}13}$ = Sow BW change between d 7 to 13 of lactation (kg/d).

$LG_{7\text{-}13}$ = Litter weight gain from d 7 to 13 of lactation (g/d).

$FI_{7\text{-}13}$ = Sow feed intake from d 7 to 13 of lactation (kg/d).

Change in sow backfat thickness was positively correlated to feed intake during the first week of lactation ($R^2 = 0.380$, $P < 0.011$) but not during the second week. There was no relationship between change in backfat thickness and litter weight gain in either week.

4.3.4 Plasma hormone and glucose concentrations

4.3.4.1 Progesterone

Plasma progesterone concentrations during farrowing ranged between 0.9 and 3.3 ng/mL and were higher in AL sows than in R sows ($P < 0.05$, see Table 4.3). After farrowing there was no treatment effect on plasma progesterone concentration (see Figure 4.3). Plasma progesterone concentration declined more rapidly during the first 6 h in AL sows than in R sows ($P < 0.01$). Feed intake on d 1 and 2 of lactation was not correlated to plasma progesterone concentrations at any time.

There was no relationship between progesterone concentration in plasma during farrowing and litter size born, but litter size was positively correlated to mean progesterone concentration on the first day of lactation ($R^2 = 0.363$, $P < 0.01$). During the two days after farrowing there were no differences in plasma progesterone concentration between the two treatments.

4.3.4.2 Prolactin

There were no differences in sow plasma prolactin concentration between treatments at any stage of lactation (see Table 4.4). Plasma prolactin concentrations were high during farrowing, 148.6 ± 10.85 ng/mL, but fell rapidly over the next 12 h to 117.1 ng/mL ($P<0.01$, see Figure 4.4). In the regression equations described later in this section d 1 prolactin (mean value for samples collected 6, 12, 18 and 24 h postfarrowing) has been used as the farrowing prolactin variable because it gave slightly higher correlation coefficients than the measurement actually taken during farrowing, however in every case farrowing prolactin concentration was also correlated to the variable.

There was a significant drop in prolactin concentration between 72 h postfarrowing and d 13 of lactation ($P < 0.001$). Mean plasma prolactin concentration on the day before weaning was 49.0 ± 2.62 ng/mL. Although there were large fluctuations in plasma prolactin concentration during the 24 h prior to weaning there was no discernible diurnal pattern (Figure 4.5).

4.3.4.3 Insulin

Plasma insulin concentrations for the two groups of sows during lactation are presented in Table 4.5. Plasma insulin concentrations were not significantly different between the two treatments during farrowing or on the first day of lactation. However by the second day of lactation AL sows had significantly greater plasma insulin concentrations than R sows, 2.28 versus 1.43 ± 0.270 ng/mL, respectively ($P < 0.05$, see Figure 4.6). Plasma insulin concentrations were consistently higher in AL sows than in R sows for the remainder of lactation. The diurnal pattern of plasma insulin concentration in the two groups of sows is shown in Figure 4.7. It can be seen that insulin concentration increased rapidly in response to feeding.

4.3.4.4 Insulin-like growth factor 1

Plasma IGF-1 concentrations for the two treatment groups are shown in Table 4.6. At farrowing there was no difference in plasma IGF-1 concentrations between sows on the two treatments, the mean value was 50.4 ± 3.28 ng/mL. However this value was negatively correlated to number of piglets born ($R^2 = 0.323$, $P < 0.02$). IGF-1 concentration increased to 54.6 ± 3.12 ng/mL within 6 h of farrowing and from then on IGF-1 concentrations were consistently higher in AL sows although this effect only became significant by d 3 of lactation ($P < 0.05$). Plasma IGF-1 concentrations declined between d 3 and d 14 of lactation ($P < 0.05$) in R sows, from 55.6 ± 6.48 to 38.5 ± 6.57 ng/mL, respectively, but not in AL sows. Hence AL sows had greater plasma IGF-1 concentrations on d 13 of lactation than R sows, 80.4 versus 38.5 ±

6.57, respectively ($P < 0.001$). Figure 4.9 illustrates the diurnal pattern of IGF-1 concentrations on d 13 of lactation and it is apparent that they remained stable over the 24 h sampling period.

4.3.4.5 Glucose

Plasma glucose concentrations were similar between treatments on the day of farrowing (Table 4.7). However, by d 3 of lactation AL sows had higher glucose concentrations than R sows ($P < 0.05$) and the magnitude of this difference had increased by d 13 of lactation with AL sows having 4.1 versus 3.5 ± 0.15 mmol/L for R sows ($P < 0.05$). In both treatments plasma glucose concentrations fell between d 1 and d 3 of lactation ($P < 0.001$) and between d 3 and d 13 of lactation ($P < 0.001$) with this effect being greater in the R sows ($P < 0.05$).

4.3.5 Piglet performance

Piglet performance data are presented in Table 4.8. There were no treatment differences in numbers born, birthweight, adjusted litter size, mortality or numbers weaned. There was no difference between treatments in piglet BW gains from birth to d 3 of lactation with all piglets averaging 128.7 g/pig/d. Likewise there was no treatment difference in piglet growth rate during wk 1. Piglets from AL sows grew faster than those from R sows in wk 2 of lactation ($P < 0.05$). Piglet daily gain in the first week of lactation was negatively correlated to plasma progesterone concentration on d 1 of lactation ($R^2 = 0.238$, $P < 0.05$), and plasma IGF-1 concentration during farrowing ($R^2 = 0.248$, $P < 0.05$). Combining these variables did not explain any more of the variance. Both of these variables were also correlated to number of piglets born. Plasma prolactin concentration during farrowing and over the first three days of lactation was positively correlated to piglet growth rate in the second week of lactation. The highest correlation was between mean plasma prolactin concentration on the day of farrowing and wk 2 piglet growth rate ($R^2 = 0.496$, $P < 0.002$). Plasma prolactin concentrations during the first three days of lactation were not correlated to piglet growth rates in the first week of lactation. Mean plasma prolactin concentration in the 24 h prior to weaning was not correlated to piglet growth rates in the second week of lactation, or during the last three days of that week. Mean sow daily feed intake was positively correlated to piglet growth rate during wk 2 of lactation ($R^2 = 0.519$, $P < 0.002$) but not during wk 1.

Litter weight gains were used as an indicator of sow milk production. Litter weight gain was similar between treatments during the first three days of lactation and unsurprisingly was positively correlated to the number of piglets in the litter ($R^2 = 0.540$, $P < 0.001$) but not to any other variables measured.

During wk 2 of lactation litter weight gain was correlated to litter size and mean plasma prolactin concentration on the first day of lactation, both of which are treatment-independent variables (Equation 4.2). Wk 2 litter weight gain was also correlated to treatment i.e. mean daily sow feed intake in wk 2 of lactation ($R^2 = 0.314$, $P < 0.02$). When treatment (feed intake) was also included in the equation it increased the amount of variance explained (Equation 4.3).

Equation 4.2

$$\mathbf{LG_{7\text{-}13} = \underset{(780.93)}{-\,2063.0} + \underset{(62.82)}{349.7\ LS} + \underset{(2.33)}{9.2\ PRL_{d1}}}$$

$$(R^2 = 0.729,\ P < 0.001)$$

Equation 4.3

$$\mathbf{LG_{7\text{-}13} = \underset{(707.75)}{-\,1698.8} + \underset{(57.54)}{315.2\ LS} + \underset{(2.73)}{5.2\ PRL_{d1}} + \underset{(54.97)}{119.9\ FI_{7\text{-}13}}}$$

$$(R^2 = 0.806,\ P < 0.001)$$

Where; $LG_{7\text{-}13}$ = Litter weight gain d 7-13 (g/d)

LS = Litter size

PRL_{d1} = Mean plasma prolactin concentration on d 1 of lactation (ng/mL)

$FI_{7\text{-}13}$ = Mean sow feed intake d7-13 (kg/d)

Therefore litter weight gain appeared to be a function of both size of the prolactin peak at farrowing and feed intake of the sow during lactation. Wk 2 litter weight gain was also correlated to plasma insulin concentration during farrowing which was also a treatment independent variable ($R^2 = 0.338$, $P < 0.02$). However this was no longer significant when feed intake and d 1 prolactin were included in the regression.

Plasma concentrations of prolactin and insulin at the end of the second week of lactation were not correlated to litter weight gain in the last week of lactation and neither were BW or fatness changes of the sows. Plasma IGF-1 concentration at the end of the second week of lactation was correlated to litter weight gain when litter size was included in the regression equation;

Equation 4.4

$$\mathbf{LG_{7\text{-}13} = \underset{(1193.32)}{-\,2592.5} + \underset{(104.74)}{451.8\ LS} + \underset{(4.25)}{9.2\ IGF\text{-}1_{d13}}}$$

$$(R^2 = 0.632,\ P < 0.004)$$

Where; $IGF\text{-}1_{d13}$ = IGF-1 concentration at the end of week 2 of lactation (ng/mL)

When feed intake was included in this regression IGF-1 concentration was no longer significantly correlated to litter gain but the variance explained by the equation increased to 90%.

4.3.6 Milk composition

Milk composition on d 5 and d 10 of lactation is presented in Table 4.9. There was no difference in protein content between milk from AL or R sows on either day of sampling. Protein content in the milk of all sows decreased from a mean value of 5.32 % on d 5 to 4.94 % on d 10 ($P<0.05$). Fat content also decreased between d 5 and d 10 ($P<0.058$). Fat content was significantly greater in the milk of R sows by d 10 than in the milk of AL sows, 6.65% versus 7.89%, respectively ($P< 0.05$). Lactose content was significantly higher in the milk of AL sows than in that of R sows on both days of sampling ($P<0.05$ d 5 and $P<0.01$ d 10). There was no change in the concentration of lactose in the milk between the two days of sampling.

Milk fat percentage on d 5 of lactation was negatively correlated to feed intake ($R^2 = 0.305$, $P < 0.05$). Feed intake ceased to be a significant factor when d 3 insulin and d 1 prolactin levels were included in the regression equation;

Equation 4.5

$$\mathbf{MF_{d5} = \underset{(0.638)}{8.74} - \underset{(0.407)}{1.59}\,INS_{d3} + \underset{(0.006)}{0.01}\,PRL_{d1}}$$

$(R^2 = 0.585, P < 0.008)$

Where;

MF_{d5} = Milk fat on d 5 of lactation (%)

INS_{d3} = Mean plasma insulin concentration on d 3 of lactation (ng/mL)

PRL_{d1} = Mean plasma prolactin concentration on d 1 of lactation (ng/mL)

Day 3 insulin is a reflection of feed intake and was used in the equation because it is the closest insulin measurement to d 5 when the milk was sampled. d 1 prolactin is a treatment independent variable.

By d 10 of lactation % milk fat was correlated only to feed intake ($R^2 = 0.469$, $P < 0.003$). Similarly d 10 milk lactose percentage was correlated to feed intake ($R^2 = 0.348$, $P < 0.02$) and equally correlated to plasma glucose concentration on d 14 of lactation ($R^2 = 0.340$, $P < 0.02$, respectively). In contrast d 5 milk lactose concentration was only poorly correlated to feed intake ($R^2 = 0.277$, $P < 0.053$) and was not correlated to plasma glucose concentration. The percentage of protein in milk on both d 5 and d 10 was not related to any of the above variables.

Milk yield was not measured in this experiment. It can be estimated by multiplying litter weight gains by 4, therefore assuming that each g of piglet growth requires 4 g of milk (Lucas and Lodge, 1961, cited by Noblet and Etienne, 1986). Using this conversion factor AL and R sows produced a mean of 8.5 versus 6.3 ± 0.87 kg milk per d in wk 1 of lactation and 11.6 versus 8.5 ± 0.81 kg/d in wk 2 ($P < 0.05$), respectively.

4.3.7 Indicators of subsequent reproductive performance

Indicators of subsequent reproductive performance are presented in Table 4.10. Both mean plasma LH concentration and the number of LH peaks occurring over a 12 h period on the day prior to weaning were significantly higher in AL sows than in R sows ($P<0.001$ and 0.01, respectively). Typical LH profiles for one sow from each group are shown in Figures 4.10 and 4.11. Only three R sows produced an LH peak during the 12 h sampling period, in each case just a single peak. This single peak occurred within 45 min of feeding in all three cases. The number of LH peaks was correlated to feed intake ($R^2 = 0.580$, $P < 0.001$) and to plasma insulin concentration on day prior to weaning ($R^2 = 0.248$, $P < 0.05$). Insulin was no longer correlated with the number of LH peaks after feed intake had been accounted for.

When the ovaries of the slaughtered sows were examined, it was found that all AL sows had follicles greater than 3 mm in diameter, with an average of 37.2 follicles in this size category per sow. In contrast only one R sow had any follicles of this size and she had just four ($P<0.001$). The number of follicles greater than 3 mm in diameter was correlated to mean plasma insulin concentration on day prior to weaning ($R^2 = 0.376$, $P < 0.01$), mean plasma IGF-1 concentration on day prior to weaning ($R^2 = 0.448$, $P < 0.01$), mean plasma glucose concentration on day prior to weaning ($R^2 = 0.540$, $P < 0.001$) and mean sow feed intake during the last week of lactation ($R^2 = 0.540$, $P < 0.001$). When these four variables were examined together IGF-1 concentration ceased to be significantly correlated to number of follicles; however, insulin concentration and feed intake or glucose concentration and feed intake together explained more of the variance in number of follicles greater than 3 mm in diameter than either one alone;

Equation 4.6

$$\mathbf{No.F} = \underset{(8.96)}{\mathbf{-\,24.4}} + \underset{(5.03)}{\mathbf{10.3}}\ \mathbf{INS_{d13}} + \underset{(2.12)}{\mathbf{6.8}}\ \mathbf{FI_{d7\text{-}13}}$$

$$(R^2 = 0.652,\ P < .001)$$

Equation 4.7 $$\mathbf{No.F = -\underset{(31.30)}{79.6} + \underset{(8.83)}{18.4}\, GLU_{d13} + \underset{(2.14)}{6.6}\, FI_{d7\text{-}13}}$$

$(R^2 = 0.655,\ P < .001)$

Where; No.F = Number of follicles greater than 3 mm in diameter.

INS_{d13} = Mean plasma insulin concentration day prior to weaning (ng/mL).

$FI_{d7\text{-}13}$ = Mean daily sow feed intake between d7-13 of lactation (kg/d).

GLU_{d13} = Mean plasma glucose concentration day prior to weaning (mmol/L).

4.4 Discussion

4.4.1 Effect of early lactation feed intake on plasma progesterone concentration after parturition

All sows had less than 4 ng progesterone/mL plasma at the time of farrowing. These values are lower than those reported for farrowing sows in Chapter 3, which averaged around 8 ng/mL, but fell within normal values reported in the literature of from 1.5 to 4.7 ng/mL (Robertson and King, 1974; Baldwin and Stabenfeldt, 1975; Duggan *et al.*, 1982). Coincidentally, since sows were not on treatment prior to or during farrowing, plasma progesterone concentration was higher at farrowing in AL sows. Plasma progesterone concentration declined more rapidly over the next 6 h in AL sows. However this is unlikely to be a treatment effect since the majority of sows had barely commenced treatment within this 6 h period. This is borne out by the fact that there was no difference between treatments in plasma progesterone concentration over the next two days and there was no correlation between sow feed intake on d 1 and 2 of lactation with progesterone concentration. It was noted in Chapter 2 that rate of decline of plasma progesterone was faster when initial concentration was higher and this is the likely explanation for the difference between treatment groups observed here. Therefore in the current experiment feed intake did not affect plasma progesterone concentration or its rate of decline during the post-parturient period. We conclude that manipulating feeding immediately postfarrowing does not increase the rate of decline of plasma progesterone.

Plasma progesterone concentration on d 1 of lactation was correlated to number of piglets born. This observation was also recorded in Chapter 3 and by de Passillé *et al.* (1993). It may be due to increased feto-placental secretion of progesterone which can occur around farrowing (Silver *et al.*, 1979) and which might be directly related to feto-placental mass.

4.4.2 Role of progesterone in lactogenesis

A decline in plasma progesterone concentrations during the periparturient period is thought to be the trigger for lactogenesis II in the sow (Hartmann and Holmes, 1989; Hartmann *et al.*, 1995). De Passillé *et al.* (1993) found that there was a negative correlation between sow plasma progesterone levels over the 48 hours after farrowing and piglet growth rates in the first three days of life. In Chapter 3 we found no relationship between plasma progesterone concentration, or its rate of decline, on piglet growth rates in the first 2 d of life. However, the more rapidly plasma progesterone concentration declined in the 6 h after farrowing, the more rapid was piglet growth rate in the first week of life. Similarly in the current experiment there was no relationship between any measure of plasma progesterone concentration and piglet weight gain in the first 3 d of life, however, there was a negative relationship between mean plasma progesterone concentration on d 1 of lactation and piglet growth rate in wk 1. This does support the suggestion that progesterone decline is necessary for the successful initiation of lactation. This relationship did not continue after wk 1 in the present experiment or in Chapter 3, and therefore rate of decline of progesterone may not be as crucial to successful establishment of lactation as are some other factors.

4.4.3 Effect of feed intake on plasma prolactin concentration in lactation

It has been reported that feeding stimulates prolactin secretion in the sow (Armstrong *et al.*, 1986; Rojkittikuhn *et al.*, 1991, 1993) and therefore we expected AL sows to have higher plasma prolactin concentrations than R sows because they received more food and were able to feed more frequently. This was not the case. There was no treatment difference in plasma prolactin concentration at any of the sampling times nor was there any correlation between feed intake and prolactin concentration. These data are in agreement with those of Baidoo *et al.* (1992) who fed sows either to appetite or 3 kg/d throughout lactation and observed no difference in plasma prolactin concentration on d 28. From the data reported in the current experiment it is concluded that manipulation of feed intake cannot be used to increase plasma prolactin concentrations during lactation.

In the current experiment plasma prolactin concentrations showed no apparent diurnal rhythm, in agreement with the observations of Bevers *et al.* (1978). In all sows plasma prolactin concentration declined as lactation progressed. This has also been reported by other workers (Armstrong *et al.*, 1986; Plaut *et al.*, 1989; Dusza and Tilton, 1990; Baidoo *et al.*, 1992).

4.4.4 Role of prolactin in lactogenesis and lactation

The prolactin peak which occurs around farrowing is known to be essential for the successful establishment of lactogenesis II (Taverne *et al.*, 1982; Whitacre and Threlfall, 1981) since if it is prevented lactation does not occur. It is not known whether the size of the periparturient prolactin peak may itself influence the efficacy of lactogenesis and the success of the resulting lactation. Two pieces of work suggest that the magnitude of the periparturient prolactin peak may be significant. Dusza *et al.* (1991, cited by Boyd *et al.* 1995) found that a single injection of purified porcine prolactin on the first day of lactation resulted in an 8% increase in litter weight gain in gilts, although not in multiparous sows. Threlfall *et al.* (1974) observed that agalactic sows had significantly lower concentrations of serum prolactin and adenohypophyseal prolactin 41 to 58 h postfarrowing than paired control sows. Both of these studies suggest that the size of the prolactin peak at farrowing may determine efficiency of lactogenesis and potential sow lactation performance. In the current experiment there was no relationship between postparturient prolactin concentration and piglet growth rate during the first week of life. This is in agreement with de Passillé *et al.* (1993)

who found mean parturient prolactin concentrations did not predict 3 d weight gain of piglets. All sows had high plasma prolactin concentrations during farrowing and presumably this, combined with the low progesterone concentrations following farrowing, was sufficient to allow lactogenesis. Mean plasma prolactin concentrations during farrowing and on the first 3 d after farrowing were positively correlated to piglet weight gain in the second week of lactation. This suggests that sow prolactin concentrations during farrowing and the first few days of lactation may influence potential lactation performance. The fact that parturient prolactin does not influence sow milk production until wk 2 of lactation signifies that it acts via modification of some fundamental mechanism which is only important as sows start to reach peak production. Unfortunately in the current experiment high prolactin concentrations in early lactation, which were independent of treatment, were confounded by subsequent treatment effects. However Equation 4.3 indicates that piglet growth rate in wk 2 of lactation was still correlated to d 1 prolactin concentrations even after feed intake had been accounted for.

In the current experiment there was no relationship between plasma prolactin concentration during established lactation and piglet growth rate. Therefore it appears that prolactin concentrations do not determine milk production during established lactation. In other monogastric species such as the rabbit (Taylor and Peaker, 1975) and the dog (Mayer and Schutze, 1973) it has been found that lactation ceases if prolactin secretion is blocked using bromocriptine or a related compound. Similar studies with sows have yielded conflicting results. Benjaminson (1981) inhibited prolactin release on d 25 of lactation, or later, but observed no corresponding decrease in piglet growth rates. He noted however that prolactin concentrations were not completely suppressed in all sows. In contrast, Farmer (personal communication) found that, regardless of the stage of lactation, piglets suffered an immediate cessation of growth following bromocriptine administration to their mother. She suggested that milk secretion was greatly, if not completely, suppressed in the absence of prolactin. Similarly, Boyd *et al.* (1995) reported that prolactin inhibition on d 29 to 30 of lactation caused a 90% drop in plasma prolactin and a 50% drop in milk yield. Therefore it seems that prolactin is necessary for maintenance of normal milk secretion in the sow.

Attempts to increase milk production by administering exogenous prolactin to sows have had limited success. Crenshaw *et al.* (1989) found no effect on milk production when sows were injected daily with 30 mg porcine prolactin i.m. from d 107 of gestation until d 2 of lactation. King *et al.* (1996) actually reduced milk production, particularly during early lactation, when they injected sows with 15 mg porcine prolactin i.m. twice daily from d 102 of gestation until weaning. Prolactin-supplemented sows had reduced protein and increased fat concentrations in colostrum compared to non-supplemented sows. This, combined with the observation that blood lactose levels rose significantly 9 d before parturition, led them to conclude that the high plasma prolactin concentrations resulting from prolactin administration had caused premature initiation of lactogenesis. Increase in blood lactose concentration has been used as a marker for the timing of lactogenesis and usually occurs within 6 h of parturition (Hartmann *et al.*, 1984). Premature lactogenesis may have caused partial involution of mammary secretory tissue because milk removal was delayed, although there was no treatment difference in mammary RNA and DNA concentrations on d 14 of lactation. Alternatively the pharmacological prolactin concentrations caused by prolactin administration in this experiment (1881 versus 91 ng/mL for prolactin-injected and buffer-injected sows respectively) may have caused down-regulation of prolactin receptors in the plasma membrane of the mammary gland (Djiane *et al.*, 1981). This would result in an apparently reduced prolactin stimulatory signal at the cellular level with subsequent reduced milk secretion.

As already mentioned Dusza (cited by Boyd *et al.*, 1995) did succeed in increasing piglet growth rates by administering prolactin on d 1 of lactation only. Likewise Cabell and Esbenshade (1990) observed increased piglet weight from d 10 and increased milk production on d 20 of lactation in response to feeding thyrotropin-releasing factor (TRF), which stimulates prolactin secretion, from d 111 of gestation until weaning. In contrast, Dubreuil *et al.* (1990) administered TRF, with or without growth hormone releasing factor (GRF), to sows subcutaneously twice daily from d 5 to 25 of lactation. Although this did result in increased plasma prolactin compared to vehicle only-treated controls there was no effect on milk production.

These observations taken together suggest that it is only when prolactin is administered within a narrow time window that it is effective in increasing milk yield. If given too early, as in the case of Crenshaw *et al.* (1989) and King *et al* (1996), prolactin supplementation may interfere with lactogenesis. The normal timing of the prolactin peak is from about 2 d before farrowing until 2 d afterwards (Taverne *et al.* 1982), therefore both groups of workers started supplementing prolactin too early and may have interfered with the normal up-regulation of prolactin receptors that occurs around parturition (Plaut *et al.*, 1989). In contrast, when increased prolactin secretion was stimulated during established lactation (d 5) this appeared to be too late to stimulate milk production (Dubreuil *et al.*, 1990). Boyd *et al* (1995) might argue that this was still too early since they recommend supplementation from d 12 of lactation to prevent stimulation of greater milk production than piglets require and the danger of causing mammary involution if milk is incompletely removed. However, when sows received additional prolactin stimulation during the immediate parturient period (Cabell and Esbenshade, 1990; Dusza, cited by Boyd *et al.*, 1995) subsequent milk production during lactation was increased. This supports our findings that the magnitude of the prolactin peak was positively correlated to piglet weight gain in the second week of lactation and appeared to be in addition to any sow feed intake effect. Cabell and Esbenshade (1990) also observed a response during the second week of lactation. However, Cabell and Esbenshade (1990) fed TRH throughout lactation as well as in the parturient period which stimulates both thyroxine and prolactin. It is equally possible that the increased growth rates they observed were due to stimulation of milk production during established lactation and not simply due to a parturient initialising. Nevertheless, comparison with the results of Dubreuil *et al.* (1990) do suggest that stimulation during the parturient period is necessary to trigger the increased production. Collier *et al.* (1984) suggested that hormone changes during the parturient period may establish the basic pattern of receptor populations in target tissues throughout lactation. As such, they would be the key to establishing a successful lactation. Our own findings certainly support this suggestion and indicate that the size of the parturient prolactin peak may determine potential milk production. In contrast the importance of prolactin during established lactation is less clear and it appears to have a permissive role rather than a deterministic role. Prolactin needs to be present for milk production to be sustained but as long as its plasma concentration remains within physiological levels it does not affect level of milk production in the sow.

In conclusion, feed intake did not affect plasma prolactin concentration in the current experiment. The magnitude of the periparturient prolactin peak was correlated to subsequent lactational performance and may be a key element in determining lactation success. Prolactin concentrations during established lactation were not correlated to lactation output, although prolactin is necessary for the maintenance of lactation. Therefore it is proposed that during established lactation, prolactin has a permissive action on milk production in the sow.

4.4.5 Role of insulin in lactogenesis and lactation

Plasma insulin concentration during farrowing was positively correlated to litter weight gain in week 2 of lactation. This suggests that, as postulated for prolactin, the concentration of insulin during the periparturient period helps to determine potential milk production. Insulin was necessary to initiate lactogenesis in *in vitro* studies with sow mammary explants (Jerry *et al.*, 1989) and therefore is probably a part of the lactogenic hormone complex in the sow. As such its concentrations during farrowing may affect basal receptor populations in target tissues throughout lactation and hence influence the whole of lactation performance (Collier *et al.*, 1984). A second and perhaps more likely explanation is that the insulin status of the sow during farrowing reflects her metabolic status and hence her ability to adapt to lactation. Unlike prolactin, the correlation between farrowing insulin and piglet growth rate was no longer significant after feed intake had been considered. Weldon *et al.* (1994a) found that sows with higher insulin concentrations in early lactation had increased voluntary feed intake in subsequent lactation. However these were sows which had been restrict fed in gestation and therefore consumed more in early lactation than sows fed *ad libitum* from d 60 of gestation and so are not directly comparable to sows in this experiment. Weldon *et al.*'s (1994a) *ad libitum* sows were also fatter at farrowing and therefore would have had higher glycerol and NEFA in the circulation which could have caused reduced appetite in lactating sows (Revell *et al.*, 1995). In the present experiment sow fatness at farrowing varied from 10 to 23 mm but there was no correlation between sow fatness and insulin concentration at farrowing.

In a second experiment, Weldon *et al.* (1994b) injected both groups of sows with insulin during lactation and found that their feed intake increased. They suggested that this was caused by a decline in glucose and lipolysis. Insulin administration also resulted in increased litter weight gain, however this was almost certainly due to the feed intake increase. Numerous experiments have shown that insulin administration only affects milk production when it induces hypoglycemia which results in decreased milk output (for reviews see Bauman and Elliot, 1983; Faulkner and Peaker, 1987). Feed intake probably increases milk production by increasing blood flow to the mammary gland. In cows and goats there is a strong correlation between rate of blood flow and milk production, and it has been observed that short-term fasting results in a rapid fall in mammary blood flow with a corresponding decline in milk production (Faulkner and Peaker, 1987). It has been suggested that insulin has a permissive role in mammary tissue with only a minimum basal concentration being required to sustain lactation (Laarveld *et al.*, 1985).

AL sows had higher plasma insulin concentrations than R sows from d 2 of lactation and also had greater piglet growth rates. However, there was no correlation between piglet growth rates and plasma insulin concentrations on the day prior to weaning ($R^2 = 0.031$, $P = 0.52$). Insulin is necessary for the maintenance of lactation (Vernon, 1989; Martin and Baldwin, 1971) and is also thought to be involved in mammary lipogenesis (Flint, 1995). Mammary lipogenesis in rats was reduced following 24 h starvation with resultant low plasma insulin concentrations and following induction of short term insulin deficiency by administration of streptozotocin (Munday and Hardie, 1987). Insulin increases both *de novo* fat synthesis in mammary tissue and also increases mammary uptake of triacylglycerides (Flint, 1995). Ironically plasma insulin concentration was negatively correlated to milk fat % on both d 5 and d 10 of lactation. This is because the dominant role of insulin is to increase glucose uptake and lipogenesis in adipose tissue. When feed intake is low insulin concentrations fall, lipolysis increases and the concentration of non-esterified fatty acids in the blood increases. NEFA are readily absorbed by the mammary tissue and incorporated into milk fat. This inverse relationship between milk fat % and feed intake has also been noted by Noblet and Etienne (1986) and Pettigrew (1995).

Lactose is the major osmolite in milk, and therefore largely determines water uptake by alveolar cells and hence milk yield (Faulkner and Peaker, 1987). Lactose is derived exclusively from glucose. Recent work indicates that glucose uptake by the mammary gland of the sow is insensitive to insulin (Hartmann *et al.*, 1995) and therefore glucose uptake continues at a similar rate regardless of insulin status (Bauman and Elliot, 1983).

4.4.6 Role of insulin-like growth factor 1 in lactogenesis and lactation

IGF-1 is both anabolic and mitogenic in a wide variety of tissues (Brameld *et al.*, 1995) and it was postulated that it may have this same role in mammary tissue during mammogenesis (Dembinski and Shiu, 1987). IGF-1 stimulated the proliferation of bovine mammary epithelial cells grown in collagen gels (McGrath *et al.*, 1991; Peri *et al.* 1992) and in ovine mammary epithelial cell clumps (Winder at al., 1989). However when IGF-1 was administered to cows via intermammary infusion from 45 d prior to due calving date there was no change in the resulting milk yield or composition (Collier *et al.*, 1992). IGF-1 has not been described as a lactogenic hormone (Denamur, 1971; Cowie *et al.*, 1980; Tucker, 1988). Mammary glands do have IGF-1 receptors which are up-regulated during lactogenesis (DeHoff *et al.*, 1988) and it has been suggested that the effects of growth hormone during established lactation may be via IGF-1 (Flint, 1995). However, repeated efforts by Flint and his group (Flint, 1995) have failed to stimulate milk production in rats by administering IGF-1, even when this was injected directly into the mammary gland. In contrast Prosser *et al.* (1994) increased milk production by 9% in goats by infusing IGF-1 directly into one mammary gland and comparing milk output with that from the non-infused gland. However, as the infusion also caused a significant increase in blood flow to the infused gland (blood flow to the non-infused gland was not measured) these results must be regarded with caution. In the current experiment sows with intrinsically higher plasma IGF-1 concentration i.e. during farrowing and on d 1 and d 2 of lactation had greater litter weight gains during the first week of lactation. Sows with higher IGF-1 status at farrowing may have been in a more anabolic state and hence better equipped for lactation. Alternatively IGF-1 may be secreted in milk and stimulate litter performance.

In dairy cows, colostrum and milk contained substantial quantities of both insulin and IGF-1 during the parturient period although these rapidly declined upon completion of lactogenesis (Malven *et al.*, 1987). It has been hypothesised that IGF-1 in colostrum and early milk will promote health and growth of the neonate (Baumrucker and Blum, 1993). During established lactation IGF-1 concentrations were correlated to litter weight gain when litter size was accounted for (Equation 4.4). Although IGF-1 was not significantly correlated to litter gain after feed intake had been included in the regression equation the resulting equation explained an additional 30% of the variance. Therefore, higher feed intake resulted in increased milk production and it appears that this effect was mediated, at least in part, via IGF-1 concentrations. This finding supports that of Pettigrew *et al.* (1993) who observed a relationship between IGF-1 concentrations during lactation and the output of milk lactose, fat and protein. Clearly this is an area which requires further investigation.

4.4.7 Milk composition and production

The hypothesis that level of feed intake would not affect milk production in the first two weeks of lactation proved to be true only during wk 1 of lactation. During wk 2 piglets from AL sows grew faster than those from R sows. Tables 4.11 and 4.12 summarise inputs and outputs of sows from the two treatments in weeks 1 and 2 of lactation, respectively. In both weeks feed intake differed markedly, as we would expect, with R sows eating 61 and 55% less than AL sows in wk 1 and 2, respectively. However the response of R sows was quite different between the two weeks. In wk 1 they mobilised more body reserves but produced similar amounts of milk to AL sows whilst in wk 2 they mobilised similar amounts of body reserves and produced less milk. Hultén *et al.* (1993) observed that restrict fed sows lost more weight in the first week of lactation than in subsequent weeks, as was observed for R sows in this experiment. R sows lost weight rapidly from farrowing throughout wk 1 and only in wk 2 were able to limit losses to the level of AL sows. In contrast AL sows actually gained weight during the first three days of lactation and overall lost only 0.4 kg/d during the first week compared to 1.0 kg/d in the second week. It is apparent from these results that feed restriction, even in the first week of lactation, when demands for milk production are at their lowest, can have a marked effect on the metabolic state of the sow and may compromise subsequent milk production.

Lactose concentration varied between treatments, although within treatment it was consistent between the two days of sampling. An increase in lactose concentration in milk from sows on higher feeding level was also observed by Smith (1960) but not by Klaver at al. (1981), although this may have been due to the small number of sows used in the latters' experiment. Lactose concentration in the milk on d 10 of lactation was correlated to plasma glucose concentration, its precursor. This finding is the opposite to that of Tokach (1991) who found no correlation between the concentration of circulating precursors and the concentration of their products in milk. Milk fat concentration was higher in R sows than in AL sows. Other workers have also observed an increase in milk fat in response to restricted feeding of the sow. (Smith, 1960; Noblet and Etienne, 1986). Noblet and Etienne (1986) observed that energy restricted sows had a lower milk yield but higher fat and protein concentrations so that nutrient output and piglet growth rate were maintained at control levels. Milk fat concentration increases in response to any manipulation which increases fat concentration in the circulation such as restricted feeding, addition of fat to the diet or increasing lysine concentration of the diet (Pettigrew, 1995). Fat and protein content both declined with advancing lactation perhaps in response to the increase in milk production, however lactose concentration remained the same within treatment - consistent with its role as an osmotic regulator.

Milk fat concentration on d 5 was positively correlated to prolactin concentration on the day of farrowing. A peak of prolactin around farrowing is essential for the onset of lactation and as already discussed there is some evidence to suggest that the higher the peak, the more successful the resulting lactation. It is apparent in the current experiment that treatment differences in milk fat content only become significant by d 10 of lactation when only feed intake was correlated to milk fatness. At d 5, whilst insulin and feed intake are already exerting some effect on milk fatness, it may be that there is still a residual effect from the priming influence of prolactin during lactogenesis. During lactogenesis prolactin regulates the synthesis of milk-specific fatty acids and the uptake of fatty acids by the mammary epithelial cells (Vonderhaar, 1987). Thioesterase II is an enzyme found exclusively in mammary tissue which is necessary for the synthesis of medium chain fatty acids and in the mouse its activity mirrors plasma prolactin concentrations (Vonderhaar, 1987). Therefore sows with greater prolactin concentrations around farrowing might have stimulated greater prolactin receptor numbers in their mammary tissue resulting in greater prolactin-stimulated milk fat synthesis on d 5.

Milk fat was negatively related to insulin and to feed intake. Milk fat may be derived from NEFA, triacylglycerides or *de novo* synthesis. Whilst it has been suggested that *de novo* synthesis of milk fat from glucose (Spincer and Rook, 1971) may be more important than incorporation of plasma-derived triacylglyceride fatty acids or NEFA in the sow (Boyd *et al.*, 1995) this will not necessarily be the case in restricted sows. Severely feed restricted animals have lower plasma insulin, glucose and IGF-1 and higher NEFA and glycerol (Baidoo *et al.* 1992) than unrestricted animals. Uptake of nutrients from the blood by the mammary tissue depends on concentration of the nutrient in the blood and rate of blood flow. If the concentration of NEFA is increased mammary cells will take up more and incorporate them into fat hence leading to greater milk fat concentration.

Pettigrew *et al.* (1993) reported a positive correlation between both insulin and IGF-I concentrations and sow yields of milk protein, fat and lactose during lactation. Unfortunately in the current experiment yields of milk components could not be calculated because sow milk yield was not measured. Although milk yield was estimated from piglet growth rates for comparative purposes, such estimates assume similar milk composition. Since milk composition varied between treatments it would be inappropriate to attempt to estimate the yield of individual components. During established lactation milk composition was not correlated to insulin or IGF-1 plasma concentrations. However, as already discussed, IGF-1 concentrations were significantly correlated to litter gain.

4.4.8 Potential subsequent reproduction

It is clear from the results that restricted feeding in the first two weeks of lactation almost completely suppressed reproductive activity. Only three R sows showed LH pulsatility, in each case just one pulse in the 12 h sampling period, and only one R sow had any follicles greater than 3 mm in diameter. This sow, which also was one of those having an LH pulse, lost less weight and backfat than other sows on the same treatment because she reared only seven piglets, whilst all the other sows reared between nine and twelve. In contrast, all AL sows exhibited both LH pulsatility and ovarian follicles greater than 3 mm in diameter even though some suffered greater loss of BW and fatness in wk 2 of lactation than some of the non-pulsatile R sows. The sow producing the greatest milk yield (an estimated 13.8 kg/d in wk 2) had the highest wk 2 BW loss of all AL sows (and higher than 5 of the 8 R sows) and considerable fat loss but she also managed to produce the greatest number of follicles greater than 3 mm in diameter (n = 64). Overall there was no difference in BW and fat loss between treatments in wk 2 of lactation despite considerable differences in feed intake and milk output. Therefore sows with similar nutrient balance during the second week of lactation had quite different metabolic hormone profiles and this was reflected in their reproductive response measured at the end of week 2 of lactation. This result reinforces Booth's (1990) observation that changes in metabolic state can occur without any obvious change in weight or fatness. Of course, in this experiment, the R sows had lost considerably more body reserves in week 1 of lactation than the AL sows.

Insulin, IGF-1 and glucose concentrations were consistently higher in AL sows than in R sows and all were correlated to the number of ovarian follicles greater than 3 mm in diameter at weaning. Cox *et al.* (1987) found that both exogenous insulin supplementation and higher dietary energy intake increased ovulation rate of gilts but the highest ovulation rates were recorded for gilts which received both insulin supplementation and higher dietary energy intake. This synergistic effect of nutrient intake and insulin concentration was also observed in the current experiment in which the number of follicles greater than 3 mm was correlated to insulin concentration even when feed intake, also positively correlated, was included in the regression analysis (Equation 4.4). Insulin may act at one or all levels of the hypothalamic-hypophyseal-ovarian axis (I'Anson *et al.*, 1991). Recent data in rams demonstrated that changes in insulin concentration in cerebrospinal fluid lead to changes in LH pulsatility, suggesting that insulin is a metabolic modulator of gonadotrophin-releasing hormone (GnRH, Miller *et al.*, 1995). Insulin levels in cerebrospinal fluid of pigs respond more slowly to change in metabolic state than plasma concentrations and are not affected by transient fluctuations in plasma insulin (Revell and Williams, 1993). In the present experiment insulin concentration was correlated to LH pulsatility but the correlation between feed intake and LH pulsatility was much stronger, suggesting that insulin is unlikely to be the only metabolic regulator involved. In Cox *et al.*'s (1987) experiment increases in ovulation rate were not necessarily accompanied by changes in LH secretion.

Glucose was also correlated to the number of follicles greater than 3 mm with or without feed intake included in the regression. Glucose might also be acting on the hypothalamic-pituitary-ovarian axis to enhance follicular development (Booth, 1990). Glucose did not appear to be involved in the regulation of ovulation rate measured by Cox *et al.* (1987) since glucose concentrations declined following insulin supplementation.

IGF-1 concentration was no longer significantly correlated to number of follicles after feed intake had been included in the regression analysis, however this does not preclude it from a role in the control of follicular development since feeding level may act via IGF-1. IGF-1 is known to stimulate growth and differentiation of both granulosa cells and thecal cells (Adashi *et al.*, 1985; Skinner and Parrott, 1994). IGF-1 is itself expressed in porcine ovaries, although not necessarily in parallel to its expression in the liver; additionally IGF-1 concentration in the circulation may not be correlated to IGF-1 concentration in follicular fluid (Charlton *et al.*, 1993).

The results indicate that restricted feeding in early lactation almost completely suppresses reproductive activity in primiparous sows. This could be particularly significant for early weaned sows. Although the sows in this experiment did not go on to a further reproductive cycle, those of Zak *et al.* (cited by Aherne *et al.*, 1995) received similar levels of feed restriction for the first three weeks of lactation. A further week of to appetite feeding was not sufficient to restore these animals to the fertility status of the control sows which had been fed to appetite throughout. Therefore it is probable that the R sows in this experiment would exhibit reduced fertility in their next reproductive cycle and they would be likely to have a significantly longer WEI than the AL sows, particularly if weaned after a 14 d lactation.

Restricted feeding in early lactation cannot be recommended for early weaned sows and has questionable value for conventionally weaned animals. Restricted sows lost most weight in the first three days after farrowing at a time when *ad libitum* fed sows were gaining weight, so that by the third day postfarrowing they were metabolically different as expressed by plasma insulin, IGF-1 and glucose concentrations. Whilst it is unlikely that commercial sows would be restricted as severely as in the current experiment it is common for sows to be tightly restricted in the first few days after farrowing, the very period during which sows in the current experiment suffered their greatest losses. Clearly this could have negative repercussions on subsequent reproductive and lactational performance.

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Table 4.1 Composition of the lactation diet

Ingredient	**Percent inclusion**
Hull-less barley	50.0
Barley	17.0
Soybean meal	20.0
Fishmeal	4.0
Tallow	5.0
Limestone	1.0
Dicalcium phosphate	1.4
Salt	0.5
Mineral/vitamin premix	1.0
DL-Methionine	0.1
Nutrient	
Digestible energy (MJ/kg)	14.42
Crude protein (%)	20.54
Calcium	0.90
Phosphorus	0.74
Lysine	1.11
Methionine + cystine	0.73 (66 % of lysine)
Threonine	0.78 (70 % of lysine)

[1] Supplied the following per kg of diet: Zinc, 120mg; Manganese, 12 mg; Iron, 150 mg; Copper, 12 mg; Selenium, 0.1 mg; Vitamin A, 5000IU; Vitamin D_3, 500 IU; Vitamin E, 22 IU; Riboflavin, 12 mg; Niacin, 45 mg; Calcium Pantothenate, 24 mg; Choline Chloride, 840 mg; Vitamin B_{12}, 30 μg; Biotin, 200 μg.

Table 4.2 Sow liveweights and backfats and their changes during a two week lactation

	Ad libitum	**Restrict**	**S.E.M.**
Farrowing liveweight (kg)	210.5	202.1	5.56
Farrowing P2 backfat depth (mm)	18.9	17.9	1.48
Weaning liveweight (kg)	200.5	178.3	5.92*
Weaning P2 backfat depth (mm)	15.9	13.6	1.12
Liveweight change from farrowing to d 7 (kg)	-2.5	-16.3	2.01***
P2 change from farrowing to d 7 (mm)	-1.3	-2.5	0.20***
Liveweight change from d 7 to 13 (kg)	-6.8	-8.3	1.67
P2 change from d 7 to 13 (mm)	-1.6	-2.1	0.42

* Significant difference between treatments ($P < 0.05$).
*** Significant difference between treatments ($P < 0.001$).

Table 4.3 Plasma concentrations of progesterone during the first three days after farrowing for sows which were fed *ad libitum* or restricted throughout lactation

Plasma progesterone (ng/mL)	***Ad libitum***	**Restrict**	**S.E.M.**
At farrowing	2.52	1.73	0.214*
24 hours postfarrowing	0.52	0.59	0.120
48 hours postfarrowing	0.27	0.45	0.073
Decline in first 6h of measurement	1.37	0.45	0.197**

* Significant difference between treatments ($P < 0.05$).
** Significant difference between treatments ($P < 0.01$).

Table 4.4 Plasma concentrations of prolactin during the first three days after farrowing and in established lactation for sows which were fed *ad libitum* or restricted throughout lactation

Plasma prolactin (ng/mL)	***Ad libitum***	**Restrict**	**S.E.M.**
At farrowing	162.4	128.5	17.70
Day 1 of lactation	131.4	100.2	13.94
Day 2 of lactation	116.0	95.6	11.80
Day 3 of lactation	107.0	95.0	8.69
Day 13 of lactation	46.7	46.1	4.20

Table 4.5 Plasma concentrations of insulin during the first three days after farrowing and in established lactation for sows which were fed *ad libitum* or restricted throughout lactation

Plasma insulin (ng/mL)	***Ad libitum***	**Restrict**	**S.E.M.**
At farrowing	1.14	0.88	0.110
Day 1 of lactation	1.62	1.21	0.242
Day 2 of lactation	2.28	1.43	0.270*
Day 3 of lactation	2.12	1.34	0.172**
Day 13 of lactation	2.29	1.12	0.107***

* Significant difference between treatments ($P < 0.05$).
** Significant difference between treatments ($P < 0.01$).
*** Significant difference between treatments ($P < 0.001$).

Table 4.6 Plasma concentrations of IGF-1 during the first three days after farrowing and in established lactation for sows which were fed *ad libitum* or restricted throughout lactation

Plasma IGF-1 (ng/mL)	***Ad libitum***	**Restrict**	**S.E.M.**
At farrowing	49.5	47.8	5.37
Day 1 of lactation	60.1	49.3	5.84
Day 2 of lactation	67.3	50.6	7.16
Day 3 of lactation	79.8	55.6	6.48*
Day 13 of lactation	80.4	38.5	6.57***

* Significant difference between treatments ($P < 0.05$).
*** Significant difference between treatments ($P < 0.001$).

Table 4.7 Plasma concentrations of glucose on days one and three after farrowing (mean of four six hourly samples) and in established lactation for sows which were fed *ad libitum* or restricted throughout lactation

Glucose (mmol/L)	***Ad libitum***	**Restrict**	**S.E.M.**
Day of farrowing	4.5	4.3	0.13
Third d postfarrowing	4.3	3.9	0.12*
Day 13 of lactation	4.1	3.5	0.15*

Table 4.8 Piglet performance from sows fed either *ad libitum* or restricted through a two week lactation

	Ad libitum	**Restrict**	**S.E.M.**
Litter size at birth	10.5	9.6	1.92
Birthweight (kg)	1.74	1.72	1.077
Litter size after cross fostering	11.0	10.7	0.75
Pigs weaned	10.4	9.7	0.56
BW gain birth to 3d (g/pig/d)	128.9	128.5	18.25
BW gain birth to 7d (g/pig/d)	201.1	161.9	17.70
BW gain 7 to 13d (g/pig/d)	276.8	218.8	16.68*

* Significant difference between treatments ($P < 0.05$).

Table 4.9 Milk composition on days five and ten of lactation for sows which were fed *ad libitum* or restricted throughout lactation

% of milk	***Ad libitum***	**Restrict**	**S.E.M.**
Day 5			
Protein	5.35	5.17	0.183
Fat	7.62	8.09	0.505
Lactose	5.17	4.73	0.123*
Day 10			
Protein	4.93	4.97	0.077
Fat	6.65	7.89	0.334*
Lactose	5.52	4.74	0.147**

* Significant difference between treatments ($P < 0.05$).
** Significant difference between treatments ($P < 0.01$).

Table 4.10 Mean luteinising hormone (LH) concentration and number of LH pulses on the day prior to weaning and number of ovarian follicles at weaning in sows which were fed *ad libitum* or restricted throughout lactation

	Ad libitum	**Restrict**	**S.E.M.**
Preweaning plasma LH concentration (ng/mL)	0.44	0.21	0.040**
LH pulses in 12 hours on d prior to weaning	3.1	0.2	0.53**
Number of ovarian follicles >3mm diameter at weaning.	37.2	0.2	4.76***

* Significant difference between treatments ($P < 0.05$).
*** Significant difference between treatments ($P < 0.001$).

Table 4.11 Sow inputs and outputs in week 1 of lactation

	Ad libitum	Restricted	SEM
Feed intake (kg/d)	4.4	1.7	0.29***
Sow BW gain (kg/d)	-0.4	-2.3	0.29***
Sow P2 backfat gain (mm/d)	-0.2	-0.4	0.03***
Litter weight gain (g/d)	2114	1587	218.1

*** Significant difference between treatments ($P < 0.001$).

Table 4.12 Sow inputs and outputs in week 2 of lactation

	Ad libitum	Restricted	SEM
Feed intake kg/d	5.1	2.3	0.32***
Sow BW gain (kg/d)	-1.0	-1.1	0.26
Sow P2 backfat gain (mm/d)	-0.2	-0.3	0.06
Litter weight gain (g/d)	2890	2128	201.7*

* Significant difference between treatments ($P < 0.05$).
*** Significant difference between treatments ($P < 0.001$).

Figure 4.1 Mean feed intake of individual sows during week two of lactation

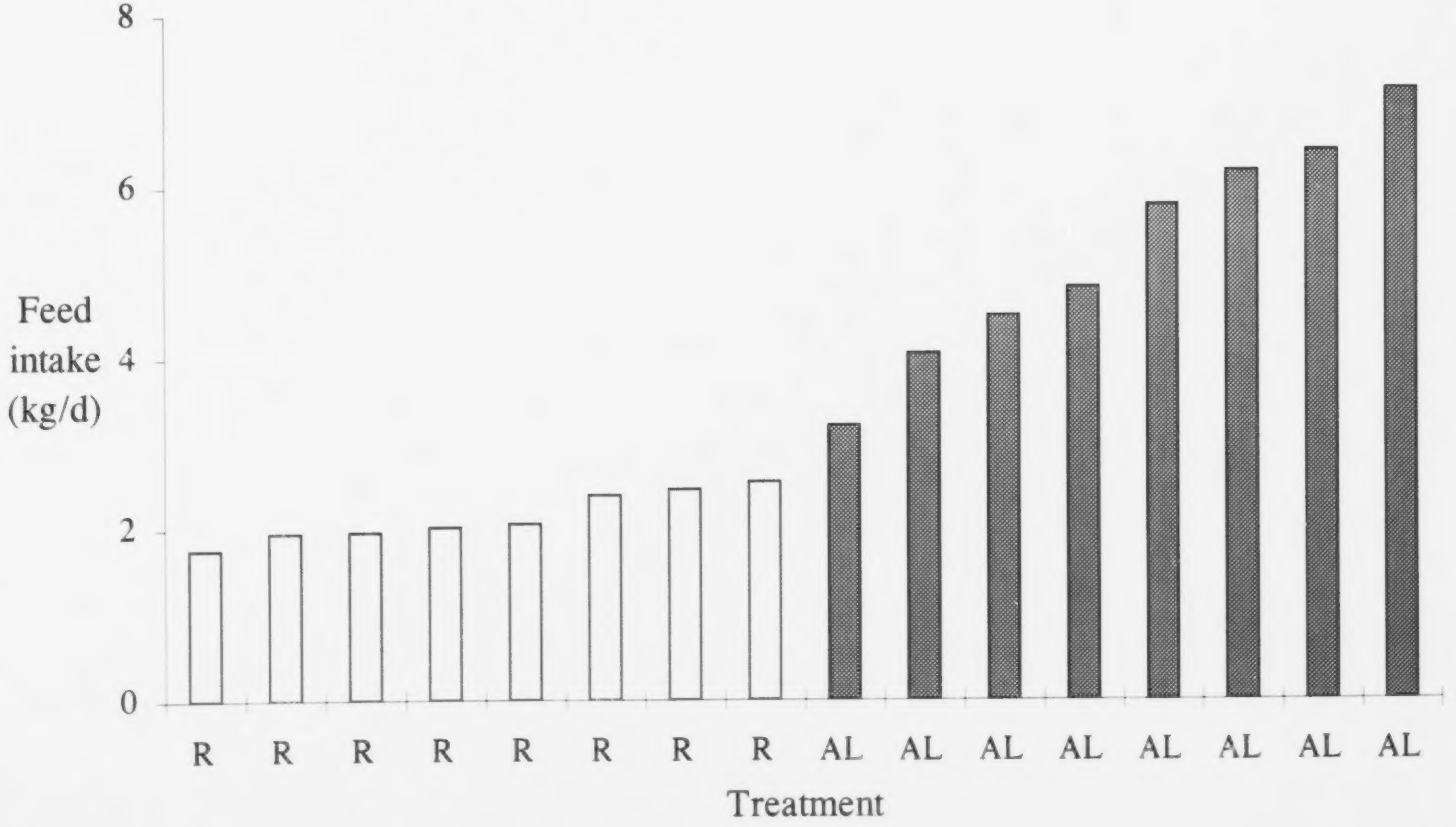


Figure 4.2 Feed intake of *ad libitum* and restricted sows during a 14 day lactation

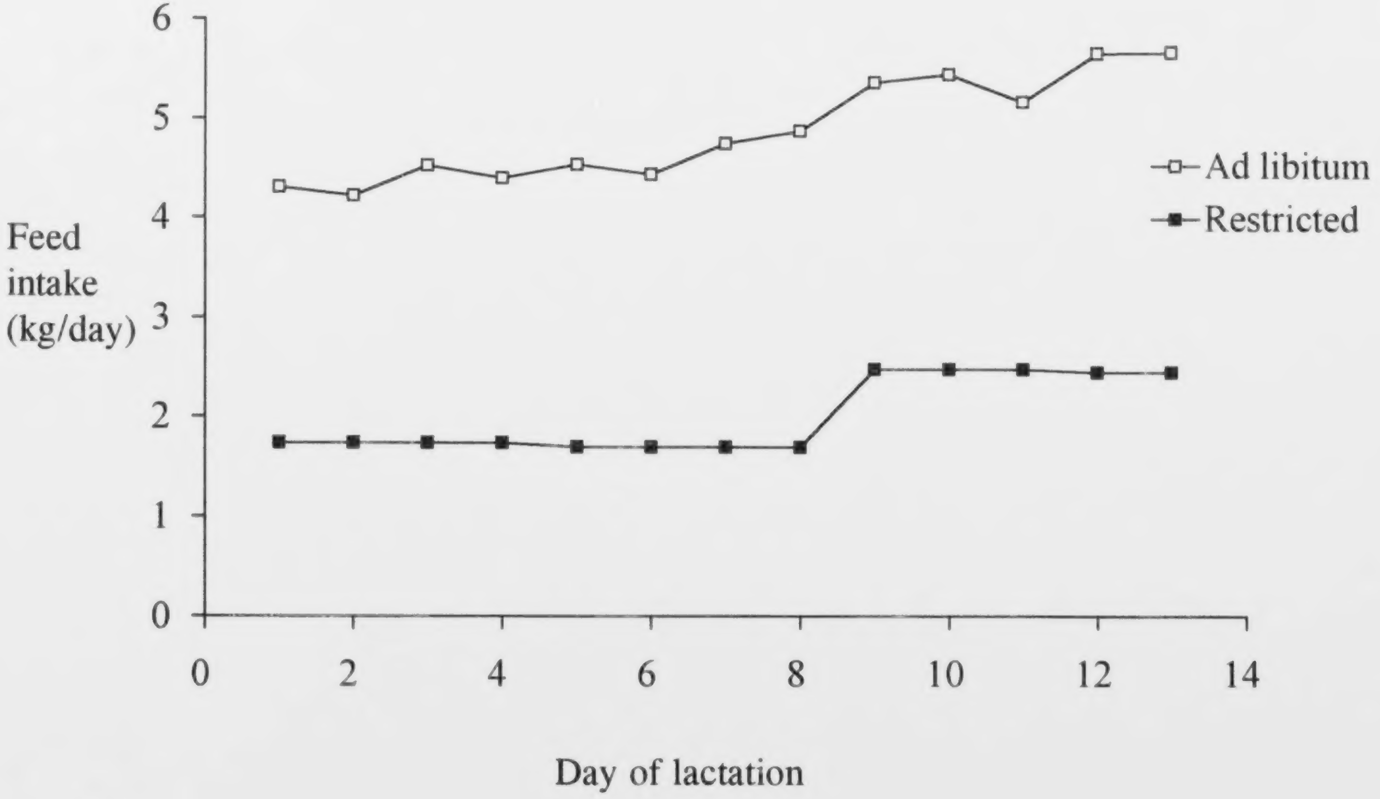


Figure 4.3 Plasma progesterone concentrations in *ad libitum* or restrict fed sows following farrowing

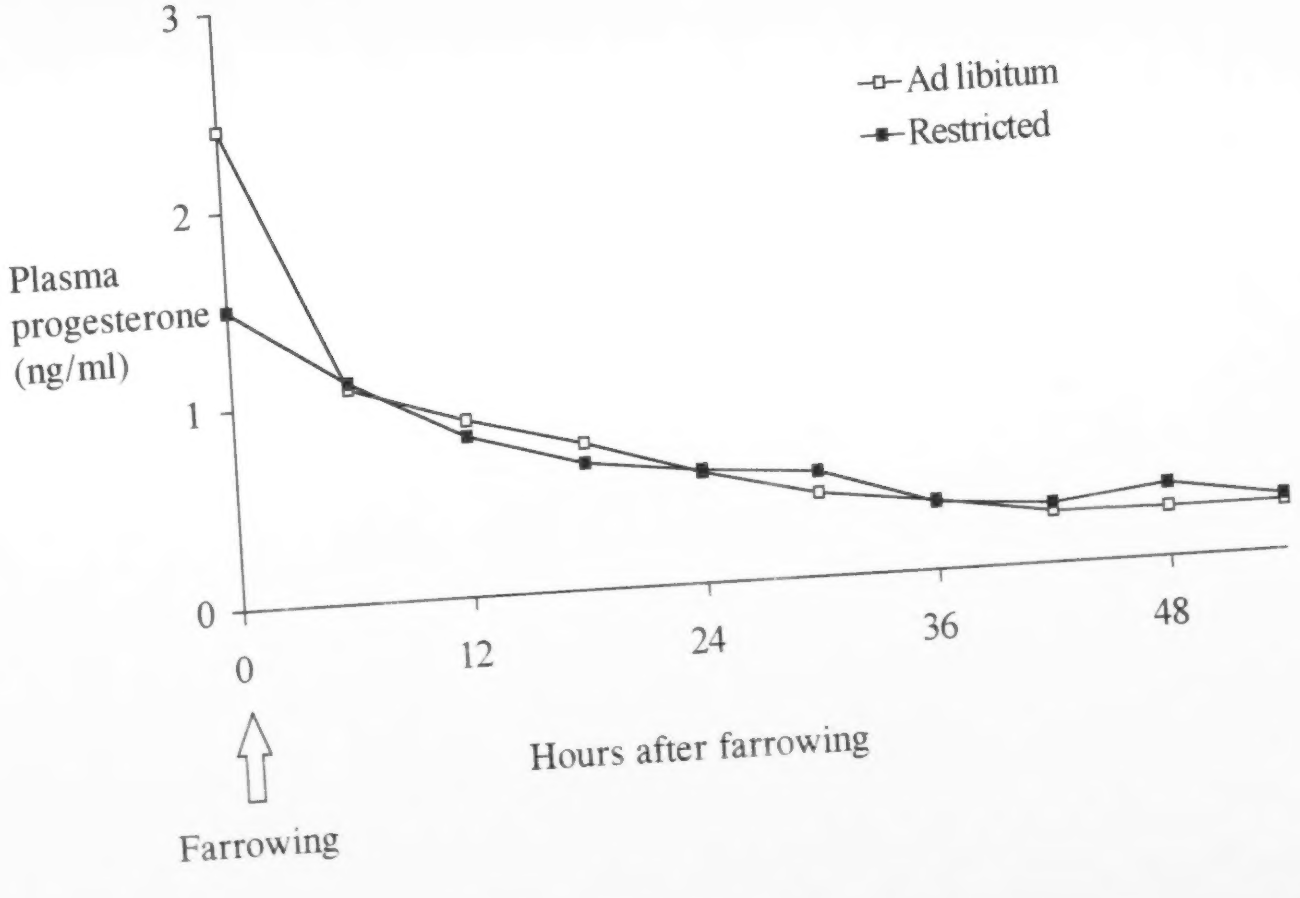


Figure 4.4 **Plasma prolactin concentrations in *ad libitum* or restrict fed sows following farrowing**

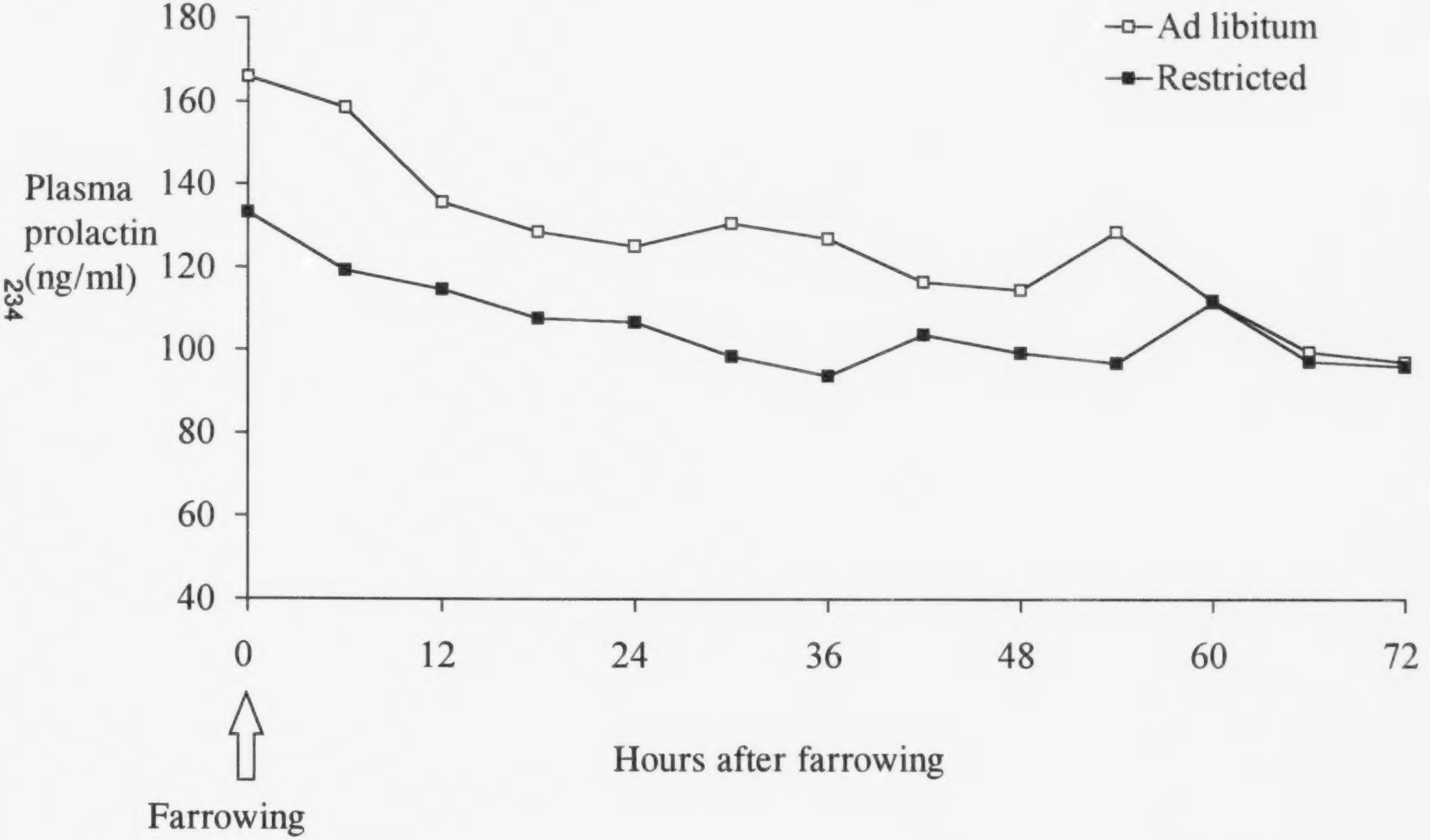


Figure 4.5 24 hour time course of plasma prolactin on the day prior to weaning for sows fed *ad libitum* or restricted during lactation

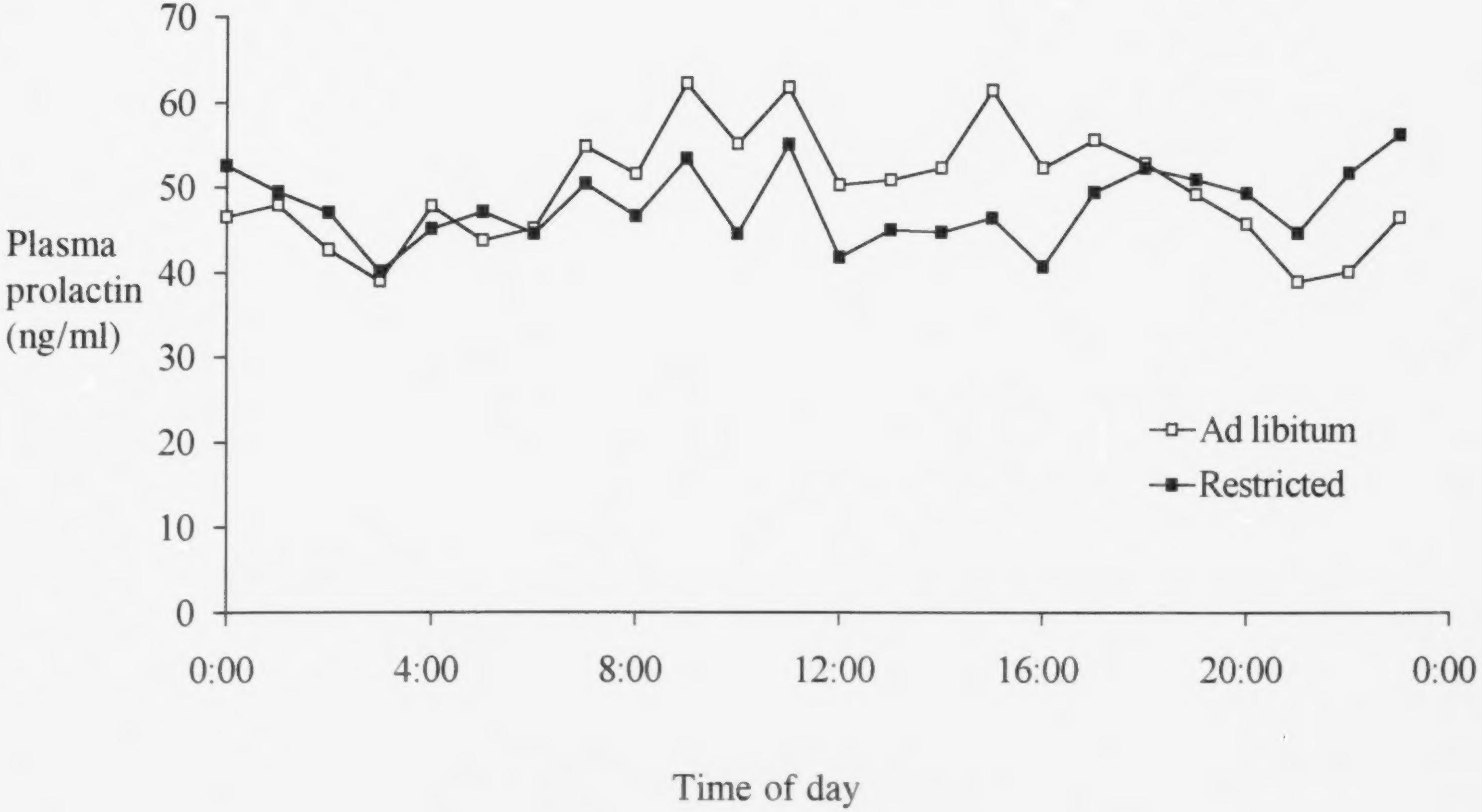


Figure 4.6 Plasma insulin concentrations in *ad libitum* or restrict fed sows following farrowing

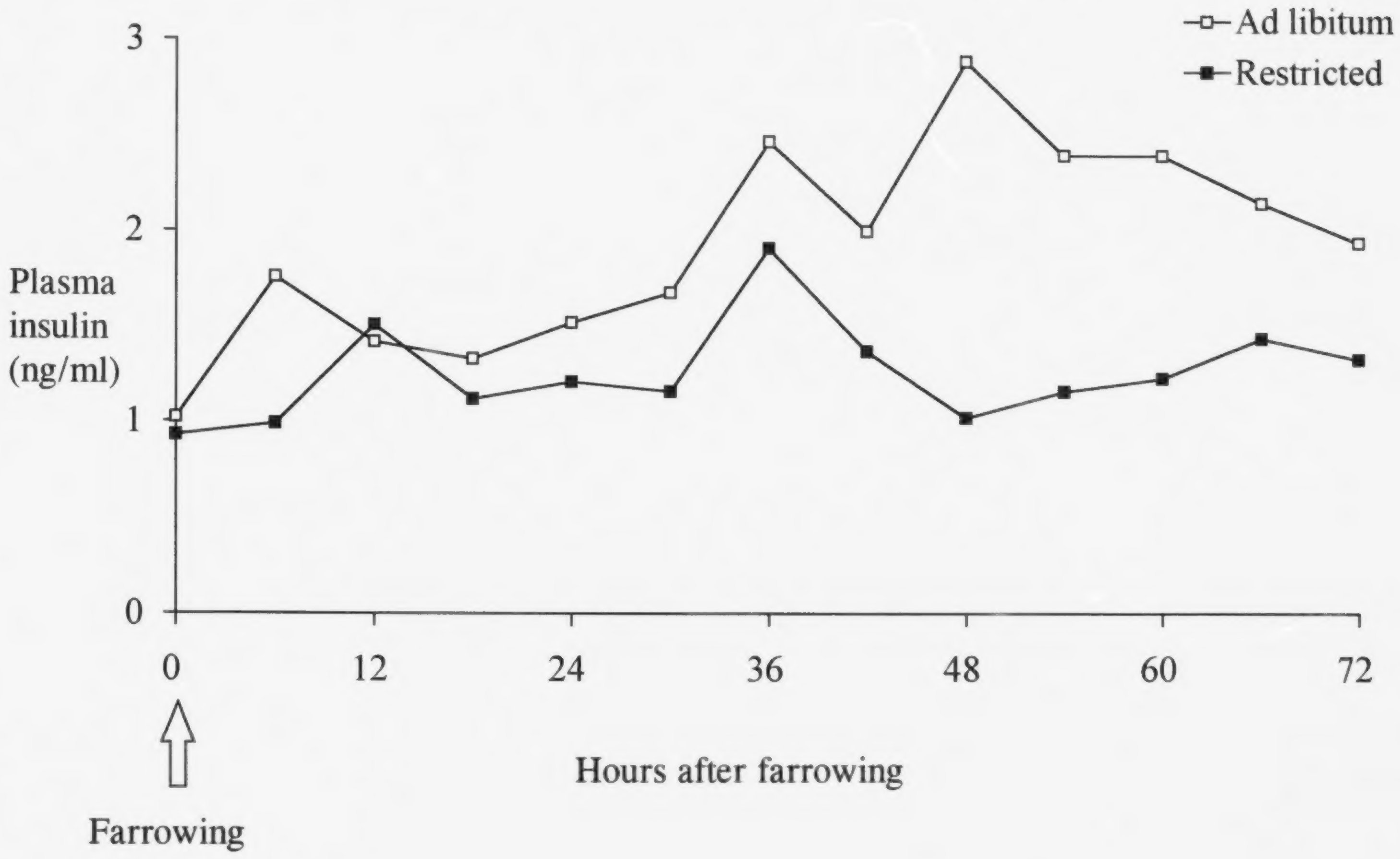


Figure 4.7 24 hour time course of plasma insulin on the day prior to weaning for sows fed *ad libitum* or restricted during lactation

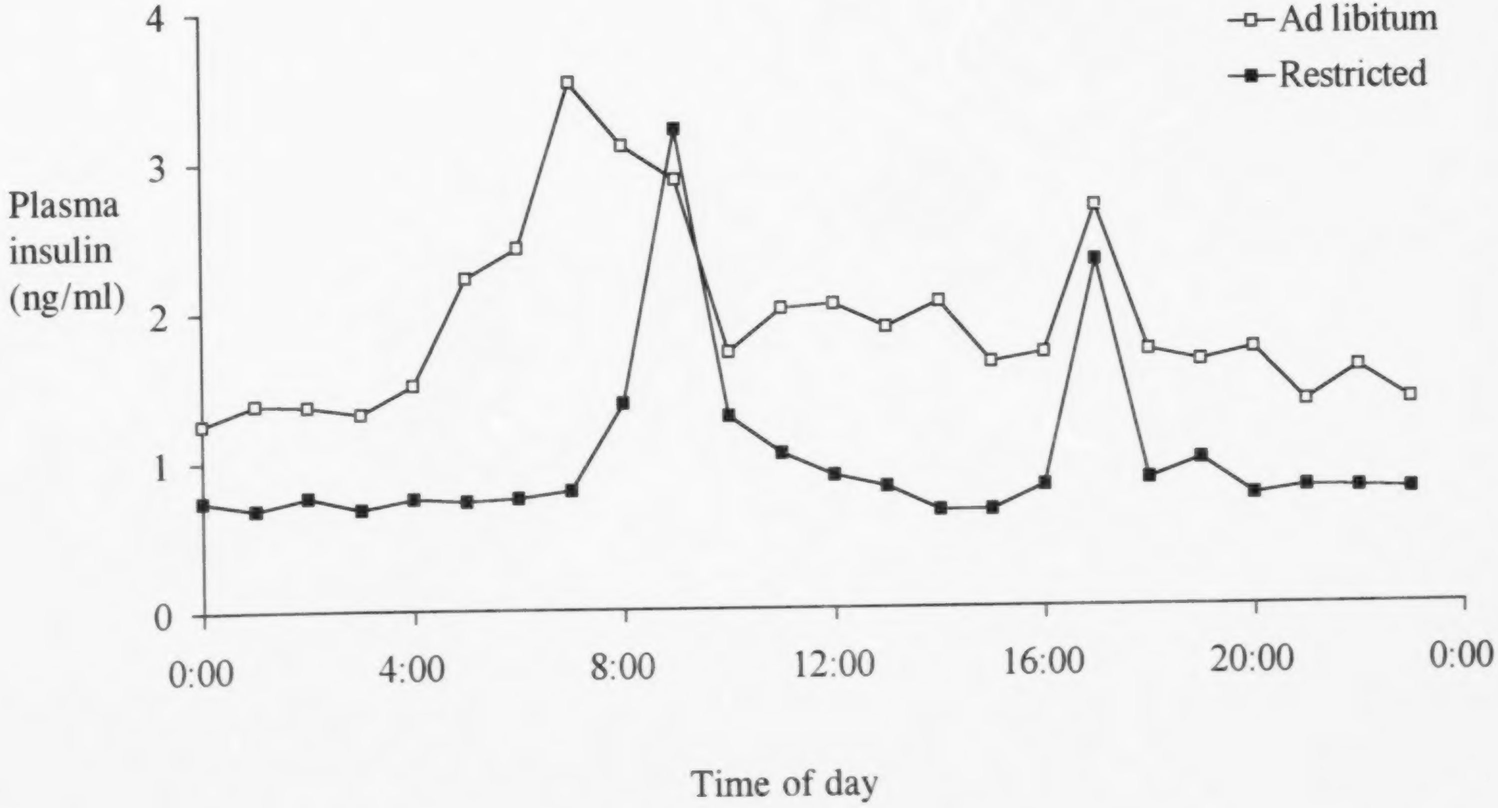


Figure 4.8 Plasma IGF-1 concentrations in *ad libitum* or restrict fed sows following farrowing

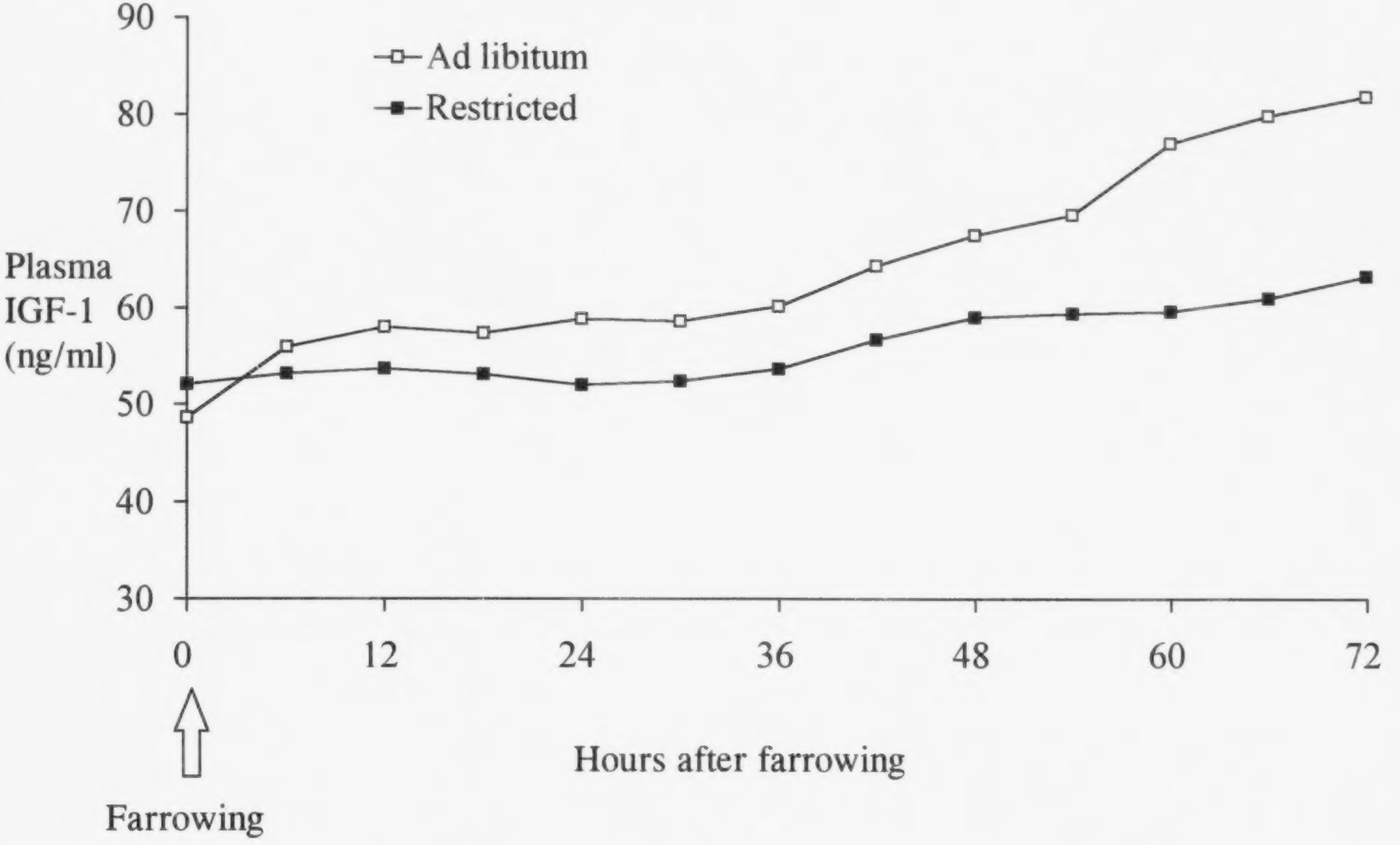


Figure 4.9 24 hour time course of plasma IGF-1 on the day prior to weaning for sows fed *ad libitum* or restricted during lactation

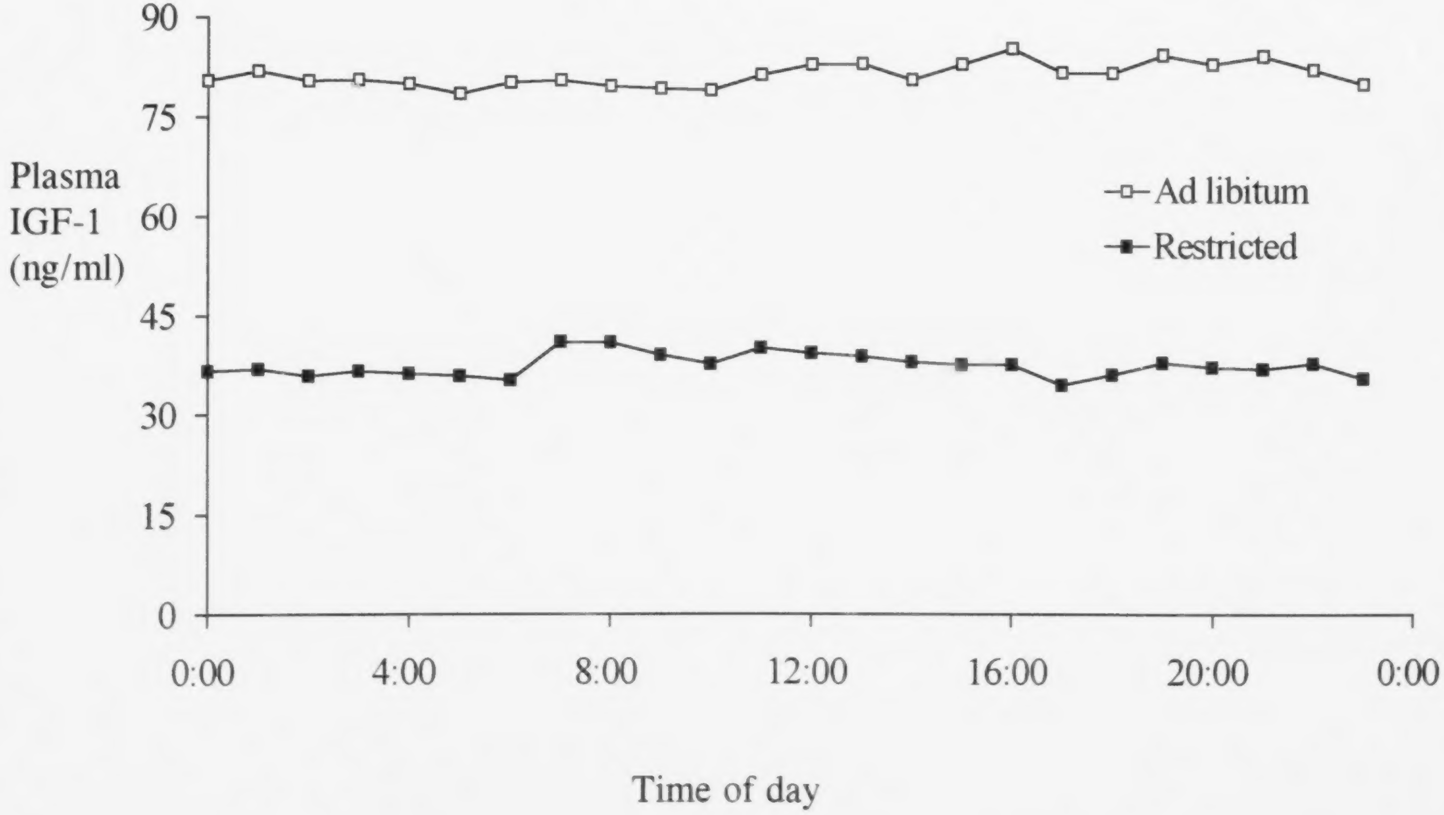


Figure 4.10 LH profile of an ad libitum fed sow on day 14 of lactation

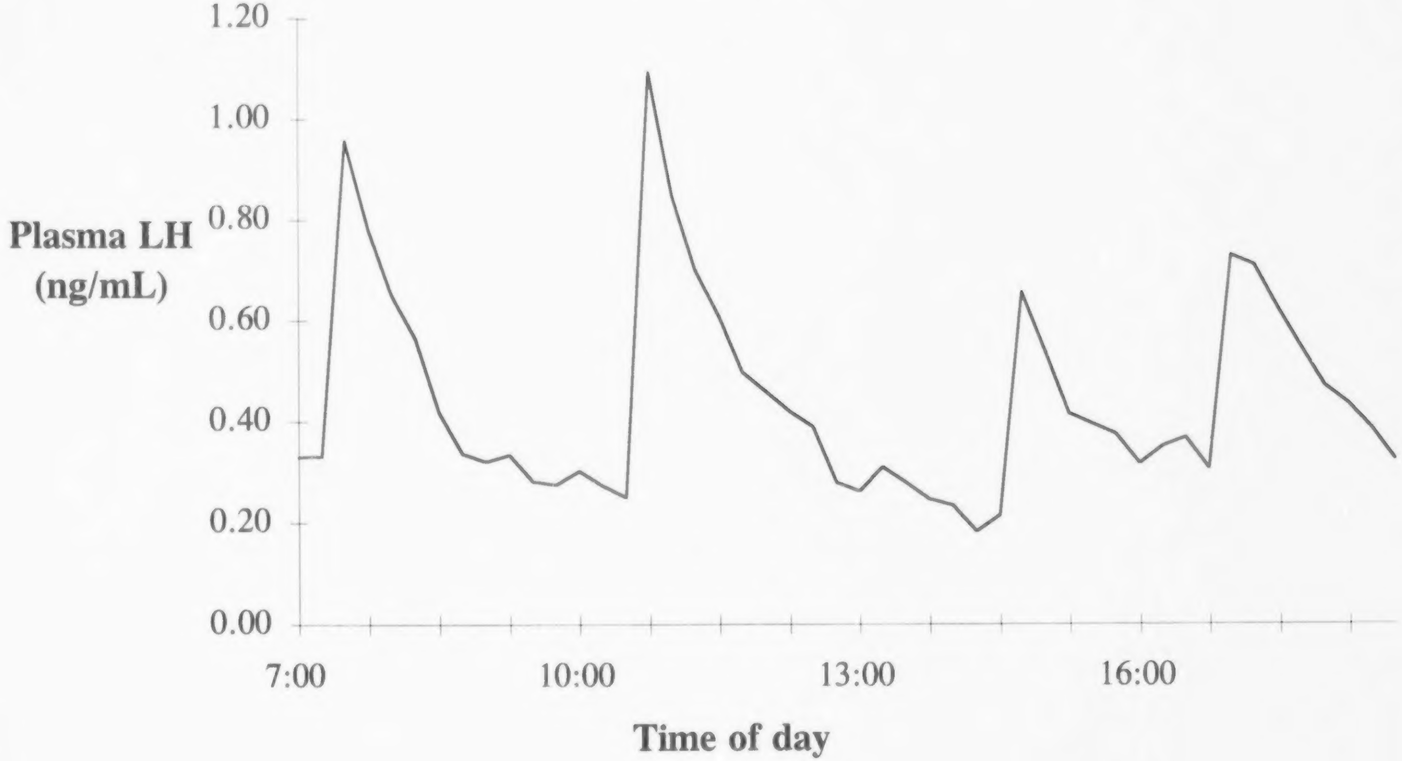
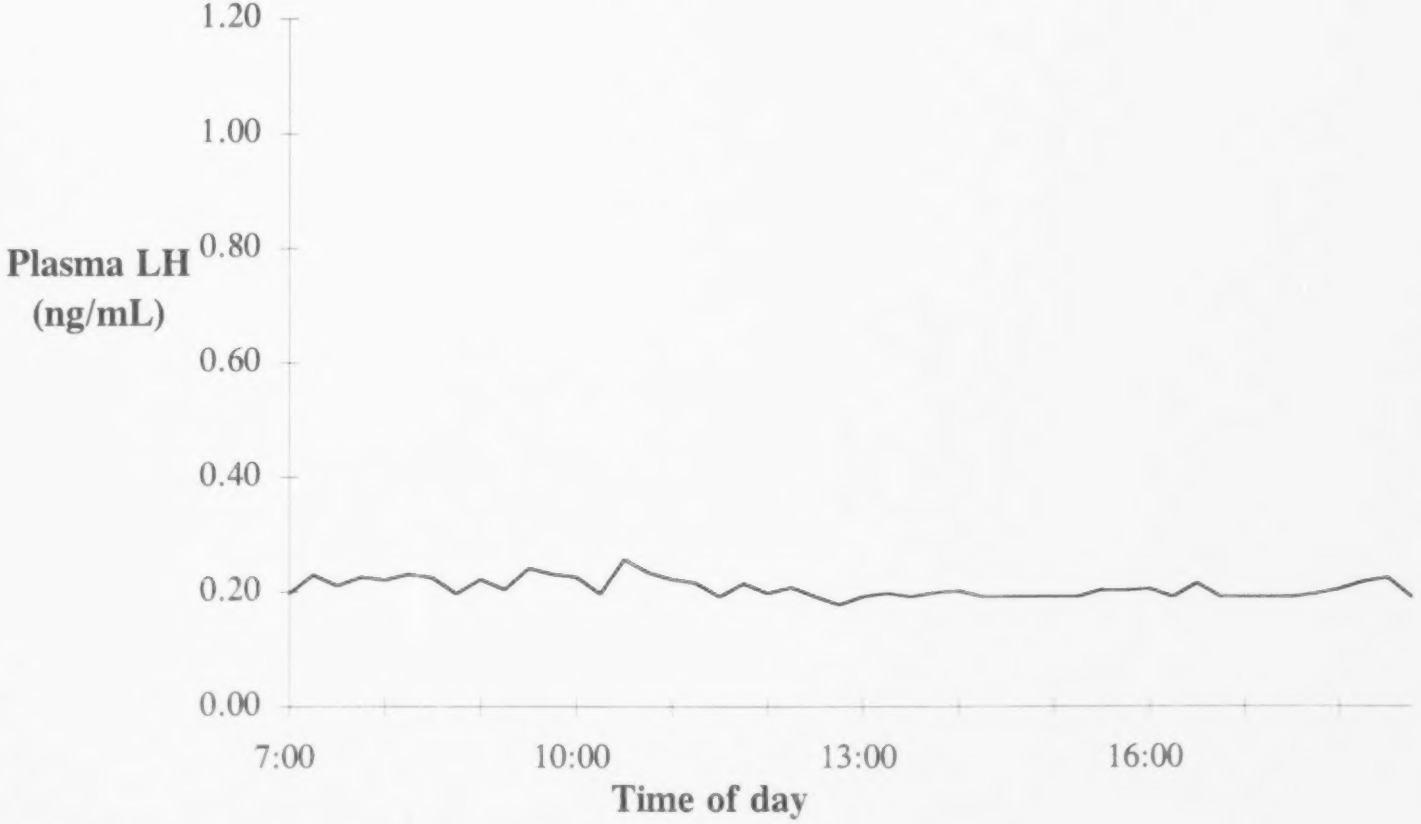


Figure 4.11 LH profile of a restricted sow on day 14 of lactation



CHAPTER 5

The Effects of Metabolic Status of Sows during Lactation on Gene Expression of Progesterone Receptor, Prolactin Receptors and Glucose Transporter in the Mammary Gland

5.1 Introduction

During lactogenesis II plasma progesterone concentrations fall and there is a peak in plasma prolactin concentrations. In rodents it has been clearly demonstrated that progesterone receptors are not present in lactating mammary tissue (Haslam and Shyamala, 1979; Kelly *et al.*, 1983) and the same appears to be true in the pig. Lin and Buttle (1991) found that progesterone receptors were present in sow mammary tissue throughout gestation but were not detectable on d 21 of lactation. One of the aims of this experiment was to determine whether these changes in progesterone receptor numbers were controlled at the transcriptional level. This was done by determining the pattern of change of progesterone receptor mRNA during late gestation and early lactation. The second experimental aim was to determine whether abundance of mRNA for progesterone receptor was related to circulating concentrations of prolactin or progesterone, prolactin receptor mRNA or piglet performance.

In contrast to progesterone receptor, mammary prolactin receptor numbers increase at the start of lactation from their levels in late gestation (Djiane *et al.*, 1977;

Emane *et al.*, 1986; Plaut *et al.*, 1989). In the rabbit this is quite dramatic, with numbers increasing by 500% in the first 6 d of lactation (Djiane *et al.*, 1977). In the sow receptor binding to ovine prolactin per gram of wet mammary tissue or per mg DNA was lowest in late pregnancy but increased two-fold by 4 d after parturition and continued to increase linearly throughout lactation (Plaut *et al.*, 1989). Plaut *et al.*'s measurements must be regarded with caution however since the work of Jerry *et al.* (1991) seriously questions using ovine prolactin as a ligand for porcine prolactin receptors. Jerry *et al.* (1991) compared the ability of ovine and porcine prolactins to bind to porcine mammary tissue and found that specific binding of ovine prolactin was much greater than that of porcine prolactin. In fact there was little or no specific binding using porcine prolactin. This difference had already been observed by Berthon *et al.* (1987) who suggested it was due to a difference in binding affinity between the two hormones. Higher binding affinity would be expected to result in a greater biological response (Jerry *et al.*, 1991) and therefore ovine prolactin should elicit a greater response than porcine prolactin. However when the ability of the two to stimulate glucose oxidation and lipogenesis in porcine mammary tissue was compared, porcine prolactin was much more potent than ovine prolactin. Jerry *et al.* (1991) attempted to explain this discrepancy by investigating the possibilities of differences in iodination and prolactin cleavage. However, a satisfactory explanation was not found and the question remains as to the nature of the target for ovine prolactin.

The increase in circulating prolactin concentrations during the periparturient period is an essential requirement for lactogenesis II to occur (Whitacre and Threlfall, 1981; Taverne *et al.*, 1982). Therefore further aims of this experiment were to determine whether mRNA for prolactin receptor was related to the level of prolactin during the periparturient period and also to establish whether mRNA followed a similar pattern of change during late gestation and early lactation to that reported for prolactin receptor by Plaut *et al.* (1989).

Inhibiting prolactin secretion during lactation causes a dramatic decline in milk production (Flückiger, 1972, cited by Mayer and Schütze, 1973; Boyd *et al.*, 1995; Farmer, Personal Communication) and therefore it is thought that higher prolactin concentrations during lactation will result in increased milk production. Attempts to achieve this by administering exogenous prolactin to pregnant and lactating sows have so far proved unsuccessful (Crenshaw *et al.*, 1989; King *et al.*, 1996) although this may be due to inappropriate timing of prolactin administration. One method of increasing prolactin concentrations in lactating sows may be to allow them *ad libitum* access to feed since Rojkittikhun *et al.* (1993) have reported that plasma prolactin concentrations were increased by feeding and declined dramatically during an overnight fast. However, as reported in Chapter 4, when the sows in this study were fed either *ad libitum* or restricted to 1.25 times maintenance energy during a two week lactation no differences in diurnal plasma prolactin concentrations or in mean plasma prolactin concentrations were observed. The *ad libitum* fed sows produced significantly more milk in the second week of lactation than the restricted sows so that there was no apparent relationship between plasma prolactin concentration and milk production.

The level of hormone stimulation that a cell receives is determined by the surrounding hormone concentration and by the number and affinity of receptors available to bind the hormone. Sakai *et al.* (1985) found that there was a relationship between the number of prolactin receptors and milk production in lactating mice. Plaut *et al.* (1989) demonstrated a positive correlation between prolactin receptors in mammary tissue explants from sows and mammary metabolic activity. They concluded that binding of prolactin to its receptor is an important effector of milk production in sows. Therefore it is likely that the *ad libitum* fed sows had greater numbers of prolactin receptors than the restricted sows, perhaps due to increased feed intake, and this resulted in greater milk yield. In this experiment the abundance of prolactin receptor mRNA in the mammary tissue of both *ad libitum* and restricted sows was determined after a two week lactation to establish whether expression of the receptor mRNA had been increased by high intake feeding.

Glucose is the sole precursor of lactose, which is the major osmoregulator in milk and therefore largely determines milk yield. It is considered that the rate of glucose uptake by the mammary gland is the main regulator of lactose synthesis (Threadgold and Kuhn, 1984) and hence the main factor determining milk production (Faulkner and Peaker, 1987). GLUT1 is the only glucose transporter that has been found in lactating mammary tissue (Burnol *et al.*, 1990; Madon *et al.*, 1990). If increasing GLUT1 concentration will increase the rate of glucose uptake and consequently milk yield, it is likely that *ad libitum* fed sows have greater concentrations of GLUT1 and that expression of GLUT1 mRNA would be up-regulated in these animals compared to the restricted sows. Therefore another aim of this experiment was to determine changes in expression of GLUT1 mRNA from late gestation, through early lactation and established lactation, and to establish whether or not the abundance of GLUT1 mRNA was correlated with milk output and feed intake.

5.1.1 Hypotheses

Based on the above discussion the following hypotheses were proposed;

- during the periparturient period there will be a decline in mRNA for progesterone receptor and a corresponding rise in the mRNA for prolactin receptor and GLUT1.
- abundance of mRNA for prolactin receptor and GLUT1 in mammary gland will be greater in sows with greater feed intake.

5.2 Materials and Methods

Sixteen primiparous Camborough sows were used in this experiment. Sows were randomly allocated to one of the following treatments;

- **AL sows** - allowed *ad libitum* access to feed throughout a 14 ± 0.2 d lactation.
- **R sows** - restricted feeding throughout a 14 ± 0.2 d lactation (R sows). These sows received 1 x maintenance energy in wk 1 of lactation followed by 1.5 x maintenance energy in wk 2 of lactation.

Animal management, surgical and blood sampling procedures and hormone, glucose and milk analyses have been described in Chapter 4. Mammary biopsies were taken from eight of the sows during the surgery required to implant jugular catheters, approximately 5 d prior to parturition, and from the remaining eight sows within 48 h of parturition.

All sows were slaughtered at a commercial abattoir within 4 h of weaning. Immediately after slaughter mammary tissue was collected from two of the five anterior pairs of glands and snap frozen in liquid nitrogen. Tissue was only collected from actively secreting glands. Mammary tissue was stored at -74 ^{o}C for 6 months prior to RNA isolation and Northern blot analysis.

5.2.1 Mammary biopsy

Sows were anaesthetised using sodium thiopental (0.17 mL/kg liveweight) and placed in dorsal or lateral recumbency. The biopsy site was prepared aseptically. The mammary tissue was exposed by a 5 cm incision perpendicular to the edge of the palpatable mammary tissue. A section of mammary tissue was isolated by blunt dissection and a 2-3 g sample excised and snap frozen in liquid nitrogen. Mammary samples were stored at -74°C until analysis. 1 mL antibacterial agent (Trimidox) was injected directly into the wound site. The incision was closed in two layers; the subcutaneous tissue was secured with a continuous suture and the skin with a continuous interlocking mattress stitch using polyglycolic acid suture. The incision site was sprayed with a topical antiseptic (Boroform). Postfarrowing biopsies were performed in the farrowing crate. Piglets were removed during the procedure after the sow had been anaesthetised.

5.2.2 Total RNA isolation

RNA was isolated from the samples using an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (1987). Briefly, each frozen mammary sample was finely ground in a pestle and mortar on dry ice. The sample was kept frozen throughout the grinding procedure by the frequent addition of liquid nitrogen. 150-200 mg of each sample, in replicates of eight, was homogenised (Ultra-Turrax homogenizer, Janke and Kunkel, Germany) in 2 mL of TRIzol reagent (Life Technologies Inc., Burlington, Ontario, Canada). The homogenised samples were incubated for 5 min at room temperature. Insoluble material was removed from the homogenate by centrifugation at 12,000 x *g* for 10 min at 4°C. The supernatant was retained and 0.4 mL chloroform added to each tube. The tubes were securely capped and shaken vigorously by hand for 15 sec prior to incubation at room temperature for 3 min. Samples were centrifuged for 15 min at 12,000 x *g* at 4°C and the aqueous phase was transferred to a separate tube. 1 mL isopropyl alcohol was added to each tube. The mixture was vortexed briefly and left to incubate for 10 min at room temperature before centrifugation for 10 min at 12,000 x *g* at 4°C. The supernatant was discarded and the RNA pellet washed with 2 mL 75% ethanol. Each sample was vortexed and centrifuged for 5 min at 7,500 x *g* at 4°C. The pellet was allowed to air dry for 10 min and resuspended in 150 mL RNase-free water. The purity of the RNA was determined by placing a RNA solution in the spectrophotometer and comparing the ratio of absorbance at 260 nm to that at 280 nm. The ratio should be around 1.8, actual ratios varied between 1.84 and 1.87.

5.2.3 Polyadenylated RNA enrichment

A polyadenylated RNA (Poly(A)$^+$ RNA) enriched fraction was prepared from the total RNA for each sample using the method of Sambrook *et al.* (1989). For each sample, 1g of oligo(dT)-cellulose (Collaborative Research Inc., USA) was placed into a 15 mL screw top tube and washed successively batchwise in 10 mL 0.1 N NaOH, 3 x 10 mL sterile water and 10 mL 1 x column loading buffer (20 mM Tris.Cl (pH 7.6), 0.5 M NaCl, 1mM EDTA (pH 8.0) and 0.1% sodium lauryl sarcosinate) after which the eluate should have a pH of less than 8.0. During each wash the oligo(dT)-cellulose was rotated gently with the solution for 5 min and centrifuged for 5 min at 900 x *g* at room temperature. All the resuspended RNA for each sample was pooled and heated to 65 °C for 5 min in a water bath. The solution was rapidly cooled to room temperature and combined with an equal volume of 2 x loading buffer. The solution was mixed with the oligo(dT)-cellulose by gentle rotation for 20 min and centrifuged for 5 min at 900 x *g* at room temperature. The oligo(dT)-cellulose was washed 5 times in 5 mL 1 x column loading buffer as described above. Following the final wash the oligo(dT)-cellulose slurry (in 1 x column loading buffer) was poured into a small column (Dispo-column, BIO-RAD, Richmond, CA, USA). Poly(A)$^+$ enriched RNA was eluted from the column using 4 x 1 mL elution buffer (10 mM Tris.Cl (pH 7.6), 1 mM EDTA (pH

8.0) and 0.05% SDS). 0.5 mL fractions were collected and their absorbance at 260 nm was measured using RNase-free cuvettes (soaked for 1 h in concentrated HCl:methanol, 1:1). The RNA peak fractions for each sample were pooled and one tenth of the pooled volume of 3 M sodium acetate (pH 5.2) was added to each. After thorough mixing, 2.5 volumes of ice-cold ethanol was added and the mixture was stored at -70 °C overnight. This mixture was centrifuged at 10,000 x *g* for 30 min at 4 °C and the resulting poly(A)$^+$ RNA pellet washed in 2 mL 70% ethanol and centrifuged at 10,000 x *g* for 10 min at 4 °C. The ethanol was decanted and the pellet air-dried for 1 h at room temperature before resuspending in 50 µL RNase-free water and storing at -70 °C.

5.2.4 Northern blotting

Poly(A)$^+$RNA was electrophoresed on denaturing 1% agarose/0.66M formaldehyde gels and transferred to Zeta-probe nylon membranes (BIO-RAD, Richmond, CA, USA) by capillary diffusion (Sambrook *et al.*, 1989). 7.5µg of each sample of Poly(A)$^+$ RNA was loaded per lane. The success of the transfer was assessed by UV-light visualisation of ethidium bromide-stained RNA on both the gel and the membrane. The positions of 28S and 18S ribosomal RNAs were marked on the membrane. Membranes were prehybridised for 2 h at 50°C in a medium containing 60% (v/v) formamide, 1 x SSPE (0.18M NaCl, 0.01 M sodium phosphate (pH 7.4) and 1mM EDTA), 0.5% (w/v) nonfat dried milk, 10% (w/v) dextran sulfate, 1% (w/v) sodium dodecyl sulfate and 500µg/mL sheared salmon testes DNA. Following prehybridisation, hybridisation was carried out for 16-20 h at 50 °C in fresh buffer containing [^{32}P]-labeled antisense RNA probes (10^6 cpm/mL). Membranes were then rinsed briefly in 2 x SSC (1 x SSC is 0.15 M NaCl and 0.015 M sodium citrate at pH 7.0), washed for 15 min at room temperature in 2 x SSC containing 0.1% sodium dodecyl sulfate, washed at 65 °C in 0.2 x SSC containing 0.1% sodium dodecyl sulfate for various times depending on the probe and rinsed briefly in 0.2 x SSC. The membranes were sealed individually in plastic bags and exposed to Kodak X-Omat AR film at -70°C with an intensifying screen in an X-ray cassette.

Relative sample loading was determined by stripping all membranes following autoradiography and rehybridising with a cDNA probe for β-actin. Hybridization with β-actin cDNA also acted as a control in comparing between mammary gland samples taken in gestation and lactation. β-actin is a housekeeping gene which is constitutionally expressed in all cells. Relative sample loading and concentration were determined by expressing all values on an equivalent β-actin basis. Membranes were washed twice for 20 min each in a large volume of 0.1 x SSC containing 0.5% sodium dodecyl sulfate at 95 °C. Membranes were prehybridised for 2 h at 65°C in a medium containing 6 x SSPE (20 x SSPE contains 3M NaCl, 0.2 M monosodium phosphate and 0.2M disodium EDTA), 0.5% sodium dodecyl sulfate, 5 x Denhardts solution (50 x Denhardts solution contains 1% Ficoll 400, 1% PVP and 1% BSA (Fraction V)) and 100μg/mL sheared salmon testes DNA. Following prehybridisation, hybridisation was carried out for 16-20 h at 65 °C in fresh buffer containing 10 mg/mL tRNA and the [^{32}P]-labeled β-actin cDNA probe (10^6 cpm/mL). The membranes were then washed 3 times for 20 min at room temperature with 2 x SSPE containing 0.1% sodium dodecyl sulfate and for 20 min at 65 °C with 0.1 x SSC containing 0.1% sodium dodecyl sulfate. The membranes were sealed individually in plastic bags and exposed to Kodak X-Omat AR film at -70°C with an intensifying screen in an x-ray cassette.

5.2.5 Probes

A radiolabeled GLUT1 antisense Riboprobe was generated from Hind III-linearised plasmid DNA [pGEM4z-hGLUT1-2 (Shows *et al.*, 1987)] using [^{32}P]cytidine triphosphate and SP6 RNA polymerase. A radiolabeled prolactin receptor antisense Riboprobe was generated from Kpn I-linearised plasmid DNA [pBluescript-rPRL-R, probe F (Boutin *et al.*, 1988)] using [^{32}P]cytidine triphosphate and T_7 RNA polymerase. A radiolabeled progesterone receptor antisense Riboprobe was generated from Kpn I-linearised plasmid DNA [pBluescript-pPR (Iwai *et al.*, 1991)] using [^{32}P]cytidine triphosphate and T_3 RNA polymerase. A radiolabeled β-actin cDNA probe was generated from β-actin DNA using random primer labeling with [^{32}P]deoxyadenosine triphosphate (Feinberg and Vogelstein, 1984). Radiolabeling of riboprobes was performed using a Riboprobe® Combination System (Promega Corporation, Madison, Wisconsin, USA) and of cDNA probes using a cDNA random primer labeling kit (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's specifications.

Transcript sizes were calculated from the log of the mass versus distance traveled by the 28S and 18S ribosomal RNAs. Masses of 28S and 18S ribosomal RNAs were assumed to be 5.1 and 1.9 kb respectively (Darnell *et al.*, 1986). Relative abundance was estimated by the absorbance intensity of bands measured on a scanning densitometer (BIO-RAD, Richmond, CA, USA).

5.2.6 Statistical analyses

Data for abundance of mRNA for progesterone receptor, prolactin receptor and GLUT1 at the end of the second week of lactation measured on all 16 sows were analysed using analyses of variance. Sources of variation were replicate, feed intake (*ad libitum* or restricted) and the interaction between them. The error term was sow within replicate by feed intake. Similar data was collected from 8 sows 5 d prefarrowing and from the other 8 sows 2 d postfarrowing. These were also analysed using analyses of variance but the sources of variation were replicate, time of measurement (-5 d or +2 d) and the interaction between them. The error term was sow within time of measurement by replicate. The difference between abundance of mRNA 2 d postfarrowing and that at the end of wk 2 of lactation for 8 sows was analysed as a split plot with sources of whole plot variance as for the model using feed intake and the split plot as two time periods (d 2 and d 14 of lactation). Although the experiment was designed to compare only two feed intake levels there was a wide range of feed intakes among the 16 sows (see Figure 5.1) and therefore multiple regression analyses were carried out to further clarify relationships between measured variables and feed intake.

Multiple regression analyses were also performed between abundance of mRNA and plasma hormone and glucose concentrations and piglet growth rate (used as an indication of milk yield). All statistical analyses were computed using Systat (Wilkinson, 1990).

5.3 Results

Hormone and production data are summarised in tables 4.2 to 4.9 in Chapter 4.

5.3.1 Effect of mammary biopsy on sow lactation

In all cases the biopsied gland continued to function normally following surgery and piglets nursing such glands grew comparably to their litter mates. Piglets did not interfere with the incision site and none of the sows showed any signs of infection following biopsy.

5.3.2 Gene expression of progesterone receptor

Northern blotting of mammary Poly(A)$^+$ RNA with a Riboprobe for progesterone receptor revealed two main transcripts of approximately 2.2 and 1.5 kb in tissue taken from sows 5 d prior to farrowing (Figure 5.2). These transcripts were smaller in size than those described for human progesterone mRNA in the human breast cancer cell line MCF-7 (Nardulli *et al.*, 1988). The 2.2 kb transcript was more intense than the 1.5 kb transcript. Both transcripts had become barely detectable by 44 h postfarrowing ($P < 0.003$) and were not detectable in the samples taken at the end of the second week of lactation. Two other faint transcripts were detected in all membranes, occurring at approximately 5.1 and 1.9 kb, and these were thought to be

28S and 18S ribosomal RNA. Abundance of the 2.2 kb transcript postfarrowing was negatively correlated to the abundance of the 4.1 kb prolactin receptor transcript ($R^2 = 0.575$, $P < 0.03$) and more variance was explained when the abundance of the 2.0 kb prolactin receptor transcript was also included in the regression, even though the latter was not correlated to progesterone receptor mRNA on its own;

Equation 5.1

$$\mathbf{P4\text{-}R_{post\ 2.2} = \underset{(0.190)}{2.06} - \underset{(0.103)}{0.45}\ PRL\text{-}R_{post\ 2.0} - \underset{(0.076)}{0.53}\ PRL\text{-}R_{post\ 4.1}}$$

$$(R^2 = 0.911,\ P < 0.002)$$

Where; $P4\text{-}R_{post\ 2.2}$ = 2.2 kb progesterone receptor transcript abundance postfarrowing.

$PRL\text{-}R_{post\ 2.0}$ = 2.0 kb prolactin receptor transcript abundance postfarrowing.

$PRL\text{-}R_{post\ 4.1}$ = 4.1 kb prolactin receptor transcript abundance postfarrowing.

5.3.3 Gene expression of prolactin receptor

Northern blotting of mammary Poly(A)$^+$ RNA with the prolactin receptor Riboprobe produced three transcripts with approximate masses of 2.0, 4.1 and 5.3 kb (Figure 5.3). In the rat prolactin receptor transcripts measured 1.8, 2.5, 3.0 and 5.5 kb (Shirota et al., 1990), however, the size of mRNAs for the prolactin receptor gene vary considerably both between species and the organs studied (reviewed by Kelly *et al.*, 1991) Table 5.1 shows the relative abundance of signal for the three transcripts 5 d pre-farrowing, 2 d postfarrowing and at weaning. The 5.3 kb transcript increased three-fold in abundance between d -5 and d 2 of lactation ($P < 0.01$), and then by a further seven-fold between d 2 and d 14 ($P < 0.001$). In contrast the 4.1 kb transcript, which was the most abundant in late gestation, had similar intensity throughout the periparturient period but increased by about the same magnitude as the 5.3 kb transcript between d 2 and d 14 of lactation ($P < 0.001$). The 2.0 kb transcript was the least abundant in late gestation, being an order of magnitude lower than the other two transcripts, and it remained at these low levels on d 2 of lactation. Between d 2 and d 14 of lactation abundance of the 2.0 kb transcript increased 85-fold ($P < 0.001$) so that there was no difference in abundance of mRNA for the three transcripts during established lactation. Abundance of the 2.0 and the 5.3 kb transcripts postfarrowing was positively correlated to plasma prolactin concentrations during the periparturient period ($R^2 = 0.59$, $P < 0.05$).

There was no treatment effect on the abundance of mRNA for any of the prolactin receptor transcripts during established lactation (see Table 5.3). Intensity of the 4.1 kb transcript during established lactation was weakly correlated to mean piglet growth rate during wk 2 of lactation ($P < 0.10$) and this became significant when mean feed intake was included in the regression;

Equation 5.2

$$\mathbf{PGR_{wk2} = \underset{(21.963)}{140.80} + \underset{(0.765)}{2.23}\ PRL\text{-}R_{L4.1} + \underset{(4.063)}{19.81}\ FI_{wk2}}$$

($R^2 = 0.709$, $P < 0.001$)

Where; PGR_{wk2} = Mean piglet growth rate wk 2 of lactation (g/d).

$PRL\text{-}R_{L4.1}$ = Abundance of 4.1 kb transcript for prolactin receptor mRNA during established lactation.

FI_{wk2} = mean feed intake wk 2 of lactation (kg/d).

During established lactation the 2.0 kb transcript was correlated to IGF-1 concentration on the day prior to weaning ($R^2 = 0.270$, $P < 0.05$) and to plasma prolactin concentration on day 1 of lactation ($R^2 = 0.284$, $P < 0.05$). However d 1 prolactin concentration and d 14 IGF-1 concentration are themselves correlated ($R^2 = 0.292$, $P < 0.05$) and when both were used together in the regression it was not significant. Abundance of the 5.3 kb transcript during established lactation was negatively correlated to plasma progesterone concentration at farrowing ($R^2 = 0.319$, $P < 0.05$).

During established lactation none of the transcripts were correlated to sow feed intake or d 14 plasma prolactin concentration.

5.3.4 Gene expression of glucose transporter 1

Northern blotting of mammary Poly(A)$^+$RNA with the GLUT1 Riboprobe produced a single transcript of 2.8 kb (Figure 5.4). This is the same size transcript as that detected by Zhao *et al.* (1993) in bovine mammary tissue. The relative abundance of GLUT1 mRNA 5 d prefarrowing, 2 d postfarrowing and during established lactation is shown in Table 5.2. Abundance tended to increase between 5 d prior to farrowing and 2 d postfarrowing ($P = 0.089$). Numerically the abundance of mRNA postfarrowing was approximately two-fold that prefarrowing, however, since only 2 prefarrowing samples could be used for this analysis, the difference was not statistically significant. Abundance of GLUT1 mRNA 2 d after farrowing was correlated to feed intake on d 1 of lactation ($R^2 = 0.608$, $P < 0.02$) and to plasma prolactin concentration during farrowing ($R^2 = 0.543$, $P < 0.05$). When these two factors were considered together both remained significant;

Equation 5.3

$$\mathbf{GLUT1_{d2} = \underset{(1.692)}{1.90} + \underset{(0.207)}{0.63}\ FI_{d1} + \underset{(0.011)}{0.03}\ P\text{-}PRL_f}$$

($R^2 = 0.861$, $P < 0.02$)

Where; $GLUT_{d2}$ = Abundance of GLUT1 mRNA 2 d postfarrowing.

FI_{d1} = Feed intake on d 1 of lactation (kg).

$P\text{-}PRL_f$ = Plasma prolactin concentration during farrowing.

Abundance of GLUT1 mRNA on d 2 of lactation was not correlated to piglet gains in the first three days of life but was correlated to piglet weight gain between d 3 and d 7 ($R^2 = 0.635$, $P < 0.02$).

GLUT1 gene expression increased between d 2 of lactation and day of weaning ($P < 0.01$), but there was no difference between treatments in amount of GLUT1 mRNA at weaning (see Table 5.3). However, abundance of GLUT1 expression at weaning was positively correlated to feed intake on d 13 of lactation ($R^2 = 0.543$, $P < 0.002$) and to piglet growth rate in week 2 of lactation ($R^2 = 0.428$, $P < 0.008$). It was not correlated to plasma concentrations of prolactin, insulin, IGF-1, or glucose either on the day prior to weaning or during the periparturient period, nor to milk composition on d 10 of lactation.

5.4 Discussion

5.4.1 Evolution of progesterone receptor mRNA during late gestation and lactation

Progesterone receptor mRNA declined dramatically between 5 d prior to farrowing and 2 d postfarrowing. Lin and Buttle (1991) determined progesterone receptor binding in mammary tissue during gestation and established lactation (d 21). Progesterone receptors were present in mammary tissue throughout gestation with the greatest concentration occurring around d 75, which coincides with the start of mammary differentiation and growth (Kensinger *et al.*, 1982). Receptor numbers declined steadily to farrowing and were undetectable on d 21 of lactation. Unfortunately mammary progesterone receptor concentrations were not measured during the periparturient period so the rapidity of decline after farrowing is not known. Data from other species suggests that the decline occurs over the first few days of lactation. In the rabbit, progesterone receptor levels were low but still detectable on d 2 of lactation but had become unmeasurable by d 10 (Kelly *et al.*, 1983) whereas in the mouse progesterone receptors were already undetectable by d 2 of lactation (Haslam and Shyamala, 1980). Haslam and Shyamala (1980) demonstrated that it is the secretory nature and/or suckling of the mammary gland which triggers the loss of progesterone receptors during lactation and not the changes which occur in the hormonal milieu. These authors thelectomised (removed the nipples from) a proportion of the glands of lactating mice whilst leaving others intact on the same animal and observed that progesterone receptor remained present in the thelectomised glands but never in the lactating glands. The mechanism by which this occurs is not known, however the results of the current experiment indicate that inhibition occurs at the transcriptional level since mRNA for progesterone receptor was barely detectable within 2 d of parturition and was not found during established lactation.

Haslam and Shyamala's (1980) observations regarding the control of progesterone receptors during lactation introduce a new concept into the regulation of lactogenesis II. It is widely believed that progesterone withdrawal is required before lactogenesis can occur in the sow (Hartmann and Holmes, 1989; Hartmann *et al.*, 1995). However, total down-regulation of progesterone receptors would have the same effect as removal of progesterone from the circulation since both would result in lack of progesterone stimulation to the mammary epithelial cell. If it is suckling itself that causes down-regulation of progesterone receptor mRNA and also causes down-regulation of existing receptors then progesterone receptor numbers will start to decline as soon as the sow starts to produce live piglets. The current experiment only tells us that gene expression for progesterone receptor was dramatically reduced in early lactation, it tells us nothing about actual receptor protein expression and turnover.

In the current experiment there was a negative correlation between progesterone mRNA and prolactin receptor mRNA 2 d postfarrowing. One of the ways in which progesterone blocks lactogenesis is by suppressing up-regulation of prolactin receptors

(Djiane and Durand, 1977). Our results support this finding and show that up-regulation of prolactin receptors probably results not only from declining plasma progesterone concentration but also declining progesterone receptor numbers within the mammary gland itself.

5.4.2 Evolution of prolactin receptor mRNA during late gestation and lactation

In 1990 Shirota *et al.* discovered that two forms of prolactin receptor were expressed in rat ovary and liver, a long-form and a short-form. It has subsequently been determined that both of these forms are also expressed in mammary tissue (Jahn *et al.*, 1991). The two forms have identical extracellular, transmembrane domains and part of the cytoplasmic domain, however the long form has a much longer intracellular domain (Kelly *et al.*, 1991). Work by Lesueur *et al.* (1991) has demonstrated that the long form stimulates β-lactoglobulin gene transcription, whilst the function of the short-form remains unknown. Both types of prolactin receptor increase in number in rat mammary tissue during lactation but although earlier work by Jahn *et al.* (1991) suggested that mRNA for the short form of the receptor predominated, more recent work by Nagano and Kelly (1994) found that the long form mRNA makes up 90% of prolactin receptor mRNA in pregnancy and 85% in lactation. Short form mRNA doubled in quantity between d 20 of pregnancy and d 7 of lactation in the rat, whilst the long-form increased only marginally over the same time period. Since prolactin has a wide variety of actions within mammary tissue which appear to be differentially regulated, it may be that the short-form prolactin receptor is the necessary link for some of these other actions. Alternatively Kelly *et al.* (1991) have suggested that the short-form may provide a means of internalising the hormone so that it can be excreted in the milk. Large quantities of prolactin are secreted in milk and it has been suggested that this confers a physiological advantage to the lactating animal (Malven, 1983, cited by Vonderhaar, 1987) perhaps by providing her with a measure of how much milk she is secreting (Bauman and Elliot, 1983).

The probe used in this experiment was derived from a rat short-form prolactin receptor (Boutin *et al.*, 1988). The probe should be able to detect both forms of prolactin receptor within the sow mammary poly(A)$^+$RNA (Jahn *et al.*, 1991) because of the shared sequence homology between the two (Kelly *et al.*, 1991). Unfortunately we do not know which of the three transcripts detected represents which form of prolactin receptor. We could speculate that the 5.3 kb transcript represents the short form because it was the only one to increase between late gestation and early lactation and this was characteristic of the short form in the rat studies of Nagano and Kelly (1994). We could also speculate that the 4.1 kb transcript is that of the long form because it was correlated to piglet growth rate and we know that long form influences secretion of milk protein (Leseuer *et al.*, 1991). However this remains speculative at the present time.

Experiments which measure prolactin binding as a means of assessing prolactin receptor numbers do not differentiate between receptor types and therefore the remainder of the discussion will concentrate on the possible implications of overall changes in mRNA for prolactin receptor. Abundance of mRNA increased between prefarrowing and postfarrowing and then increased further with advancing lactation. Prolactin regulates its own receptor numbers (Forsyth, 1983). Increase in circulating prolactin concentrations results initially in down-regulation of prolactin receptors (Djiane *et al.*, 1979) due to increased internalisation and lysosomal breakdown (Djiane *et al.*, 1981). Longer term, increases in prolactin concentration cause up-regulation of prolactin receptors in mammary tissue (Forsyth, 1983; Kelly *et al.*, 1991). Surprisingly, up-regulation of prolactin receptors in mammary tissue also occurs following inhibition of prolactin secretion with bromocriptine (Djiane *et al.*, 1977). Plasma prolactin is high during farrowing and this may have stimulated the increased gene expression of the 5.3 kb transcript on d 2 of lactation compared to levels in late gestation. The other two transcripts had not increased above prefarrowing levels by d 2 of lactation. However, gene expression of both the 2.0 and the 5.3 kb transcripts on d 2 of lactation was positively correlated to plasma prolactin concentration during the periparturient period suggesting that prolactin is having a stimulatory effect on receptor production in early lactation.

As lactation progressed plasma prolactin concentrations declined whilst milk yield increased from an estimated average of 5.5 kg/d in the first 3 d of lactation to 10.3 kg/d in wk 2. These changes were accompanied by a dramatic increase in mRNA for prolactin receptor which, if translated into actual receptor numbers would guarantee that the prolactin signal at the mammary gland would be maintained or increased despite the falling prolactin concentration in the circulation. These findings are in agreement with the changes observed by Plaut *et al.* (1989) in receptor binding. However, they are exactly opposite to the theory that prolactin regulates its receptors in parallel with its own concentration (Djiane and Durand, 1977). In this experiment gene expression of prolactin receptor was up-regulated as prolactin concentration decreased. In addition there was no correlation between plasma prolactin concentration during the 24 h prior to weaning and abundance of prolactin receptor mRNA in mammary tissue sampled within 4 h of weaning. This suggests that prolactin, at least within the range of plasma concentrations measured in this experiment, does not regulate its own receptors during established lactation.

There was no treatment difference in prolactin receptor mRNA at the end of the second week of lactation, nor any correlation between feed intake and abundance of prolactin receptor mRNAs, therefore prolactin receptor gene expression was not augmented by high intake feeding. AL sows had greater milk output in wk 2 of lactation than R sows. There was a weak relationship between abundance of the 4.1 kb transcript and piglet growth rate. Feed intake was strongly correlated to piglet growth rate. However inclusion of abundance of 4.1 kb transcript in the regression equation explained more of the variance without reducing the level of significance (see Equation E5.2), suggesting that the increased milk yield of the high intake sows was primarily due to feed intake but that this effect was modulated by prolactin receptor numbers. Plasma prolactin concentrations were unaffected by treatment and were not correlated to piglet growth rate so the observed effect appears to be via increased prolactin binding. This is in agreement with Plaut *et al.*'s (1989) observation that mammary metabolism was correlated to prolactin binding.

Abundance of the 5.3 kb transcript at the end of wk 2 of lactation was negatively correlated to plasma progesterone concentration during farrowing. This does support the theory that progesterone concentrations during the periparturient period may be important in determining successful establishment of lactation (de Passille *et al.*, 1993). However, in the current experiment variation in abundance of the 5.3 prolactin receptor transcript was not reflected in any difference in piglet performance.

5.4.3 Evolution of glucose transporter 1 mRNA during late gestation and lactation

Glucose is the sole precursor of lactose which is the major osmoregulator in milk and therefore to a large extent determines milk yield. It is considered that the rate of glucose uptake by the mammary gland is the main regulator of lactose synthesis (Threadgold and Kuhn, 1984) and hence the main factor determining milk production (Faulkner and Peaker, 1987). GLUT1 is the only glucose transporter that has been found in the epithelial cells of lactating mammary tissue (Burnol *et al.*, 1990; Madon *et al.*, 1990; Zhao *et al.*, 1993; Camps *et al.*, 1994). In the current experiment GLUT1 mRNA in mammary tissue increased approximately two fold in abundance between 5 d prefarrowing and 2 d postfarrowing and then a further two fold between d 2 and the end of the second week of lactation. This two fold increase in expression of GLUT1 mRNA between d 2 and the end of wk 2 of lactation is of the same magnitude as the estimated increase in milk production which occurred over the same time period (from 5.5 kg/d d1-3 of lactation to 10.3 kg/d in wk 2). Assuming that GLUT1 protein expression is determined by abundance of mRNA levels then this supports the idea that glucose transport into the mammary gland is strongly related to milk production. Further support for this hypothesis was the positive correlation between GLUT1 mRNA and piglet growth rate in both wks 1 and 2 of lactation.

The temporal changes we observed in abundance of GLUT1 mRNA in sows were different from those described in rats by Burnol *et al.* (1990), who noted an overall fall of about 40% in mRNA abundance between parturition and peak lactation. This apparent difference may be because they did not correct their data with a housekeeping gene to allow for the diluting effect of mRNA for milk proteins in peak lactation (Jahn *et al.*, 1991)

Although there was no treatment difference in the abundance of GLUT1 at the end of wk 2 of lactation, expression of GLUT1 was positively correlated to feed intake both in early lactation and at the end of wk 2. GLUT1 mRNA on d 2 of lactation was also correlated to plasma prolactin concentration during farrowing and this relationship remained even after feed intake had been considered. This finding supports the hypothesis put forward in Chapter 4 that the height of the prolactin parturient peak may help to determine the success of the subsequent lactation. Flint (1995) concluded that both prolactin and growth hormone are necessary to fully maintain the level of GLUT1 in mammary tissue. Prolactin deficiency caused a 35% reduction in number of GLUT1 transporters in lactating rat mammary gland (Fawcett *et al.*, 1991) whilst a lack of growth hormone caused a reduction of 20% (Flint, 1995). Growth hormone was not measured in the current experiment, however whilst gene expression of GLUT1 was related to prolactin concentration in early lactation, during established lactation there was no relationship between plasma prolactin concentration and GLUT1 mRNA. Likewise there was no relationship between gene expression of prolactin receptors and that of GLUT1. Therefore if circulating prolactin does alter glucose transporter levels during established lactation then this is not controlled at the transcriptional level.

In this experiment GLUT1 mRNA was significantly correlated to feed intake but not to any of the metabolic hormones measured. Both insulin and IGF-1 did increase in higher intake sows, however. Various studies have shown that glucose transport in lactating mammary gland is not acutely stimulated by insulin (Laarveld, *et al.*, 1981, 1985; Prosser and Topper, 1986; Munday and Williamson, 1987) and this has also been confirmed in the sow (Holmes, 1991, cited by Hartmann *et al.*, 1995). Insulin and IGF-1 do cause up-regulation of GLUT1 transcription in both mammary and other tissues following prolonged *in vitro* exposure of tissue explants (Prosser and Topper, 1986; Prosser *et al.*, 1987; Kahn and Flier, 1990). Therefore, it is possible that the up-regulation of GLUT1 mRNA observed in this experiment may have been caused by prolonged exposure to increased concentrations of these two metabolic hormones. Alternatively it may be the increased concentration of plasma glucose itself which has stimulated up-regulation of GLUT1. Low extracellular levels of glucose resulting from glucose starvation have been shown to increase GLUT1 expression in other tissues (Gould and Holman, 1993) but the converse has not been reported.

5.5 Conclusions

The results of this experiment support the inverse relationship between progesterone concentration and prolactin receptor numbers during lactogenesis as indicated by prolactin receptor mRNA. Progesterone receptor mRNA fell dramatically within 2 d of parturition suggesting that this is a rapid response controlled at the transcriptional level. The role of prolactin in control of its own receptors and GLUT1 transporters during established lactation is questioned; if such control does exist it is not at the transcriptional level. Prolactin receptor mRNA increased between d 2 and 14 of lactation and was negatively related to changes in plasma prolactin concentration occurring over the same time period. GLUT1 mRNA increased in parallel with sow milk production thus supporting the theory that rate of glucose entry into mammary cells is the primary determinant of milk yield. Expression of GLUT1 may be related to insulin, IGF-1 or glucose concentrations in the circulation since all were increased by *ad libitum* feeding but GLUT1 expression was only correlated to feed intake itself in established lactation. Prolactin receptor mRNA was also correlated to GLUT1 expression in early lactation.

5.6 References

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Table 5.1 Relative abundance of mRNA for prolactin receptor pre and postfarrowing and during established lactation

Transcript	Prefarrowing	Postfarrowing	Lactation
PRL-R 2.0 kb	0.20^{a} (0.213)	0.22^{a} (0.174)	18.7^{b} (2.73)
PRL-R 4.1 kb	2.40^{a} (0.297)	2.27^{a} (0.243)	17.5^{b} (2.32)
PRL-R 5.3 kb	1.09^{a} (0.425)	3.25^{b} (0.347)	21.0^{c} (2.47)

Figures with different superscripts within rows are significantly different ($P < 0.05$).

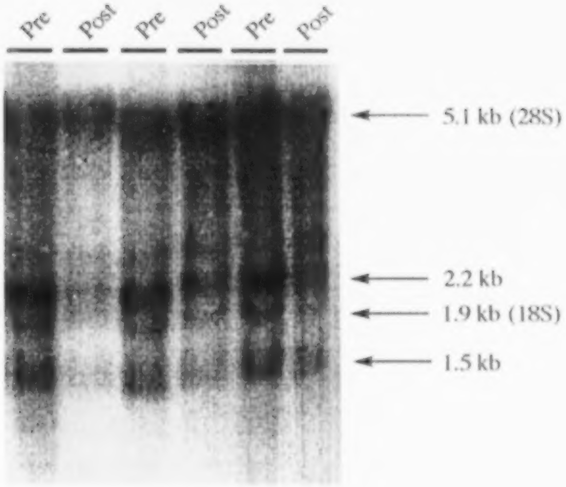
Table 5.2 Relative abundance of mRNA for GLUT1 pre and postfarrowing and during established lactation

Transcript	Prefarrowing	Postfarrowing	Lactation
GLUT1	6.79 (2.656)	13.01 (1.533)	33.85 (5.513)

Table 5.3 Relative abundance of mRNA for prolactin receptor and GLUT1 during established lactation in sows which were fed *ad libitum* or restricted during lactation

Transcript	***Ad libitum***	**Restricted**	**SEM**
PRL-R 2.0 kb	22.84	14.02	4.602
PRL-R 4.1 kb	18.05	15.28	3.961
PRL-R 5.3 kb	22.39	18.00	3.406
GLUT1 2.8 kb	38.39	22.19	8.280

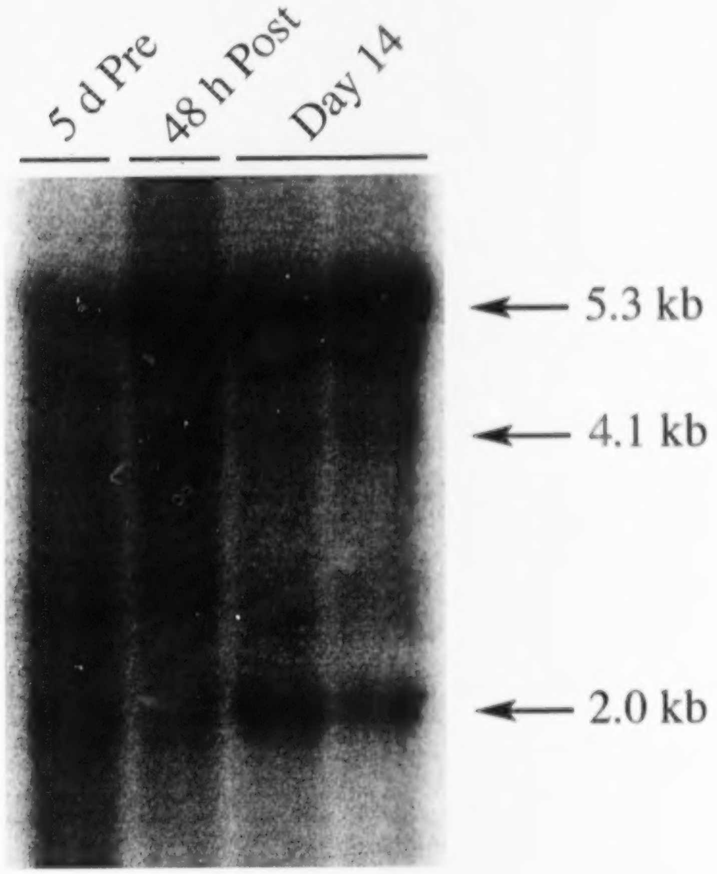
Figure 5.1 Progesterone receptor mRNA in sow mammary tissue 5 days prefarrowing and 2 days post farrowing



Northern blots performed with 7.5 μg poly (A^+) RNA from pregnant or lactating sow mammary gland were hybridised for progesterone receptor as outlined in the materials and methods. Membranes were washed for 15 min at room temperature in 2 x SSC plus 0.1% SDS followed by 15 min at 65°C in 0.2 x SSC plus 1% SDS. Autoradiograms were exposed for 24 h.

Size of transcripts are shown. 5 d Pre = sample taken 5 d before farrowing, 48 h Post = sample taken 2 d after farrowing.

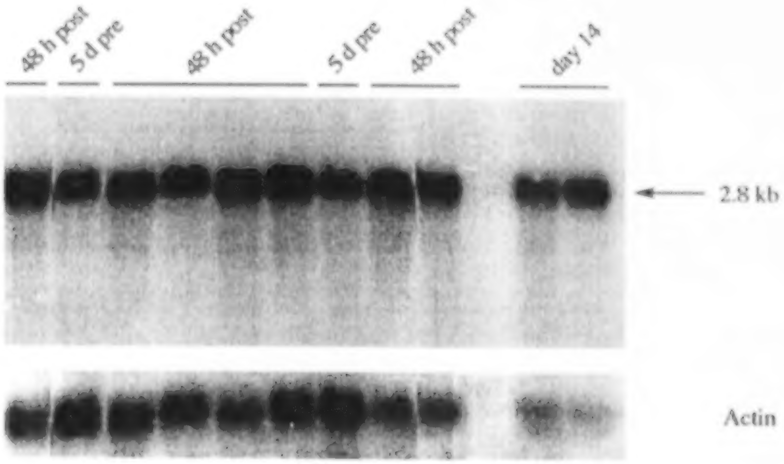
Figure 5.2 Prolactin receptor mRNA in sow mammary tissue 5 days prefarrowing, 2 days post farrowing and at the end of the second week of lactation



Northern blots performed with 7.5 μg poly (A^+) RNA from pregnant or lactating sow mammary gland were hybridised for prolactin receptor as outlined in the materials and methods. Membranes were washed for 15 min at room temperature in 2 x SSC plus 0.1% SDS followed by two washes of 15 min each at 65°C in 0.2 x SSC plus 1% SDS. Autoradiograms were exposed for 48 h.

Size of transcripts are shown. 5 d Pre = sample taken 5 d before farrowing, 48 h Post = sample taken 2 d after farrowing and d 14 = sample taken at the end of the second week of lactation.

Figure 5.3 Glucose transporter-1 mRNA in sow mammary tissue 5 days prefarrowing, 2 days post farrowing and at the end of the second week of lactation



Northern blots performed with 7.5 μg poly (A^+) RNA from pregnant or lactating sow mammary gland were hybridised as outlined in the materials and methods. Following the GLUT1 hybridisation membranes were washed for 15 min at room temperature in 2 x SSC plus 0.1% SDS followed by three washes of 20 min each at 65°C in 0.2 x SSC plus 1% SDS. Autoradiograms were exposed for 24 h. Following the β-actin hybridisation membranes were washed three times for 20 min each at room temperature with 2 x SSPE plus 0.1% SDS and for 20 min at 65°C with 0.1 x SSC plus 0.1% SDS. Autoradiograms were exposed for 3 days.

Size of transcripts are shown. 5 d Pre = sample taken 5 d before farrowing, 48 h Post = sample taken 2 d after farrowing and d 14 = sample taken at the end of the second week of lactation.

CHAPTER 6

General Discussion and Conclusions

The periparturient period is a time of dynamic change for the sow as she switches from progesterone-dominated placental nutrient transfer during pregnancy to prolactin-dominated mammary nutrient output during lactation. Throughout this period the sow's demands for nutrients are steadily increasing. Firstly to meet the increasing demands for fetal growth during late gestation and then to provide an increasing volume of milk for the growing piglets as lactation proceeds. The overall aim of this thesis was to investigate how sows responded to different levels of feed intake during the periparturient period and to investigate the regulation of progesterone and prolactin during lactogenesis and lactation.

6.1 Effect of feed intake in late gestation

In late gestation, sows are relatively unaffected by feed intake and seem to be able to maintain normal fetal growth rates regardless of nutrient input. Increasing sow feed intake or changing the quantity and quality of nutrients supplied during late gestation has little effect on piglet birthweight or body reserves at birth (Hillyer and Phillips, 1980; Pettigrew, 1981; Cieslak *et al.*, 1983; Campion *et al.*, 1984; Bishop *et al.*, 1985ab; Stirling and Cline, 1986; Coffey *et al.*, 1987; Newcomb *et al.*, 1991). This was confirmed in Chapter 3 where increasing feed intake during the last two weeks of gestation did not affect piglet birthweight. Even extreme measures such as causing the sow to develop diabetes resulted in only marginal improvement in piglet status at birth (Ezekwe and Martin, 1978; Ezekwe, 1986) and seem to mainly benefit piglets which would otherwise have been disadvantaged. Failure to increase sow feed intake in late gestation was shown to cause sows to become catabolic and to lose backfat prior to farrowing. However, as reported in Chapter 3, this did not affect the ability of sows to adapt to lactation. Lactation feed intake, diet digestibility and piglet performance were similar whether sows received 1.15 or 2.0 times maintenance during late gestation. The major benefit of increased feeding in late gestation was the prevention of backfat loss prior to farrowing. Such sows should be better able to buffer nutritional inadequacies in lactation. Levels of sow body reserves at farrowing can affect her lactational and subsequent reproductive performance if feed intake is restricted during lactation (Mullan and Williams, 1989).

When sows received higher feed intakes in late gestation for only one parity there was no difference in lactation or subsequent reproductive performance (Chapter 3; Hillyer and Phillips, 1981; Stirling and Cline, 1986). However, when high feed intake in late gestation was provided for several successive parities (Cromwell *et al.*, 1989) there were improvements in piglet birthweight and sow performance after sows had been on treatment for at least two parities. Therefore, although the sow is able to buffer inadequate nutrient intake within one parity without apparent detriment, it is better for her long-term performance and longevity not to allow her to fall into energy deficit prior to farrowing.

6.2 Feed intake effects on lactogenesis II

Lactogenesis II requires falling progesterone concentrations combined with high prolactin concentrations in the presence of insulin (Hartmann *et al.*, 1995). In sheep and pigs there is an inverse relationship between feed intake and plasma progesterone concentration (Cumming *et al.*, 1975; Dyck *et al.*, 1980). In addition, progesterone is extremely lipophilic (Hillbrand and Elsaesser, 1983). Therefore manipulation of sow fatness and feed intake during the periparturient period might influence the rate of decline of progesterone following farrowing and hence the timing of lactogenesis II. Progesterone-infused, ovariectomised, pubertal gilts were used to evaluate the effects of feed intake and body fatness on the kinetics of progesterone metabolism (Chapter 2). In this model, feed intake significantly increased the MCR of progesterone with corresponding reductions in plasma progesterone concentrations and a tendency to shorten progesterone half-life. Surprisingly, body fatness affected neither MCR of progesterone nor rate of decline of progesterone after farrowing. The response to feed intake appeared to offer some potential for manipulating plasma progesterone concentrations around farrowing in the sow. However, when sows were offered high or low feed intakes in late gestation (Chapter 3) or early lactation (Chapter 4) there was no effect on plasma progesterone concentrations. However, in contrast to the gilt, sow fatness did affect the rate of decline of progesterone postfarrowing with fatter sows having longer progesterone half-lives. Increases in feed intake or sow fatness did not cause a delay in lactogenesis, as determined from piglet weight gains in the first two days of life and therefore cannot be used to manipulate timing of lactogenesis II. In addition it was concluded that the progesterone-infused ovariectomised gilt was not an appropriate model in which to study progesterone kinetics of the pregnant or lactating sow.

6.3 Hormonal control of lactogenesis II

Most work on the onset of lactogenesis II has been performed with rodents. It has been assumed that similar control mechanisms operate in the sow but little definitive work to support this conclusion has been carried out. Several authors have reported negative relationships between plasma progesterone concentration and plasma or milk lactose concentration (Martin *et al.*, 1978; Willcox *et al.*, 1983; Holmes and Hartmann, 1993). De Passillé *et al.* (1993) observed a negative correlation between mean plasma progesterone concentration in the 48 h after farrowing and piglet growth rate in the first three days of life. The work in this thesis supports these earlier studies since plasma progesterone concentration in the postfarrowing period was negatively correlated to piglet growth rate in the first week of life. This finding is in agreement with the hypothesis that rapid progesterone withdrawal following farrowing is associated with better establishment of lactation. A new finding was that expression of progesterone receptor mRNA was negatively correlated to that of prolactin receptor mRNA. Progesterone is believed to suppress lactogenesis in part by suppressing up-regulation of prolactin receptors (Tucker, 1988). The present work suggests that down-regulation of progesterone receptors as well as decline in plasma progesterone concentration may act together to remove the progesterone block to lactogenesis.

High levels of prolactin at farrowing are also necessary for successful lactogenesis II (Taverne *et al.*, 1982). However, the data presented in Chapter 4 led to the hypothesis that the size of the prolactin peak itself may influence the sow's lactation potential. Plasma prolactin concentrations during and after farrowing were significantly correlated to piglet growth rate in the second week of lactation and continued to explain a significant proportion of the variance even when feed intake was also included in the regression equation. Unfortunately this effect was confounded with feed intake in lactation since the high prolactin sows coincidentally had been allocated to the high intake treatment. However, Equation 3.14 defines the poor correlation between sow feed intake and piglet growth to 20 d normally observed in the University of Alberta swine herd. The literature also supports this hypothesis in that attempts to increase lactation output by administering exogenous prolactin or by stimulating increased endogenous production with growth hormone releasing factor have only been successful when administration commenced close to farrowing (Crenshaw *et al.*, 1989; Cabell and Esbenshade, 1990; Dubreuil *et al.*, 1990; Dusza, cited by Boyd *et al.*, 1995; King *et al.*, 1996). Nevertheless, further investigation is required to confirm this hypothesis.

6.4 Effect of feed intake during the first two weeks of lactation

In the first two weeks of lactation sows are still quite tolerant of feed deficiency and low feed intakes generally only start to affect piglet growth rate from the third or fourth week of lactation (King and Dunkin, 1985; Mullan and Williams, 1989; Yang *et al.*, 1989). In the experiments reported in Chapters 3 and 4, there was no effect of feed intake on piglet growth rate during the first week of lactation. The relationship between sow feed intake and piglet growth rate to 20 d of age was shown to be weak and numerically small in Chapter 3 (Equation 3.14).

Sow feed intake does not affect piglet growth rate in the first week of lactation and yet there is wide variation in piglet growth rate between sows. Much of this variation can be attributed to the birthweight of the piglet (Mullan and Williams, 1989; Van der Steen and de Groot, 1992). Equation 3.11 (Chapter 3) clearly shows that piglet birthweight was the major determinant of piglet growth rate in the first week of life with a small advantage to piglets from later parity sows, perhaps as the result of larger gland size in older sows. There was also a decrease in piglet growth rate in piglets from larger litters. This effect was probably underestimated since larger litters generally consisted of lighter pigs. Therefore, in the first week of lactation milk output was largely determined by the vigor of the sucking piglets. Sows were able to meet piglet demands and were able to buffer inadequate nutrient intake.

In contrast to the low correlation between feed intake and piglet growth rate up to three weeks of age observed in Chapter 3, milk output was shown in Chapter 4 to be strongly correlated to feed intake during the second week of lactation with *ad libitum* sows having significantly greater litter weight gains than restricted sows. This effect of feed intake may have been mediated via IGF-1 concentrations since, of all the hormones measured, only IGF-1 concentrations were correlated to litter gain during wk 2 of lactation. It has been suggested that plasma prolactin concentrations will be increased by increasing sow feed intake (Rojkittikuhn *et al.*, 1993), however, this was not observed in the present experiment (Chapter 4). Prolactin is the dominant hormone of lactation and yet during established lactation not only was there no relationship between feed intake and prolactin concentration but also there was no relationship between plasma prolactin concentration and milk production (see Chapter 4). It was hypothesised that this disparity might be explained by different numbers of prolactin receptors in the mammary tissue of high producing sows. The novel approach of investigating abundance of prolactin receptor mRNA was used to investigate this question (see Chapter 5). One of the prolactin receptor mRNA transcripts was shown to be correlated to piglet growth rate (see Equation 5.2) and this effect was in addition to that of feed intake, suggesting that receptor expression modulates the effect of feed intake in the mammary gland. This is in agreement with the *in vitro* studies of Plaut *et al.* (1989) who observed that mammary metabolism was correlated to prolactin binding. In the present study up-regulation of prolactin receptor mRNA was not correlated with feed intake, prolactin concentration, IGF-1 concentration or insulin concentration. Therefore the increased milk production resulting from higher feed intake does not appear to be mediated via increased prolactin stimulation.

During the research conducted for this thesis, GLUT1 mRNA was identified for the first time in sow mammary tissue. It was found that abundance of GLUT1 mRNA increased in parallel with milk production. In addition, during both week 1 and week 2 of lactation, milk yield and feed intake were correlated with the expression of GLUT1 mRNA in mammary tissue. This is consistent with the role of glucose as a determinant of milk yield (Faulkner and Peaker, 1987). GLUT1 expression (was not correlated to) did not appear to be regulated by insulin or IGF-1 in this experiment, although concentrations of both metabolic hormones were elevated in the high yielding *ad libitum* sows. GLUT1 regulation is generally regarded to be independent of insulin regulation (Laarveld, *et al.*, 1981; Prosser and Topper, 1986; Munday and Williamson, 1987). However, both insulin and IGF-1 caused up-regulation of GLUT1 in rat mammary tissue following prolonged incubation of mammary explants (Prosser and Topper, 1986; Prosser *et al.*, 1987; Kahn and Flier, 1990). Although the mechanism of GLUT1 regulation was not elucidated in the present experiments, it seems that one of the ways in which feed intake increases milk production is via up-regulation of GLUT1.

6.5 Periparturient feed intake and subsequent reproductive performance

Feed intake during the periparturient period only affected subsequent reproductive performance when the nutrient restriction was imposed during lactation.

Feed intake in late gestation had no apparent effect on subsequent reproductive performance (Chapter 3), whereas feed restriction during a two week lactation resulted in almost total suppression of reproductive activity (Chapter 4). It has been hypothesised that changes in the metabolic state of the sow at any time during follicular development may have lasting effects on the quality of the resulting follicles (Foxcroft *et al.*, 1995). However, feed restriction has more profound negative effects on subsequent reproductive performance the more closely it is associated with the time of weaning (Zak *et al.*, reported by Aherne *et al.*, 1995; Koketsu *et al.*, 1996). In Chapter 3, feed intake in week 3, but not in weeks 1 or 2, of lactation was significantly correlated to litter size in the subsequent parity (Equation 3.15). Therefore, feed restriction in the two weeks immediately before weaning would be expected to have a greater impact on subsequent reproductive performance than reducing feed intake 4 to 6 weeks prior to weaning, as was observed. In addition, the degree of restriction during lactation (Chapter 4) was far more severe than that imposed during late gestation (Chapter 3) due to the higher nutrient requirements associated with milk production. *Ad libitum* fed sows lost more backfat in a two week lactation than low intake sows lost during the last two weeks of gestation, 2.9 mm versus 1.6 mm. The effects of feed restriction appeared to be mediated by insulin, in line with the work of Cox *et al.* (1987) and Matamoros *et al.* (1990, 1991). IGF-1 may also have been involved since its concentration was elevated in *ad libitum* fed sows and it is known to stimulate follicle development (Adashi *et al.*, 1985; Skinner and Parrott, 1994). Therefore, this work (Chapters 3 and 4) supports the hypothesis that feed restriction is more detrimental the more closely it is associated with time of farrowing. Feed intake in late gestation is likely to have a greater effect on subsequent reproductive performance of early weaned sows. Feed restriction in early lactation suppressed the resumption of reproductive activity following farrowing (Chapter 4) and this is likely to have lasting effects on subsequent reproductive performance (Foxcroft *et al.*, 1995).

6.6 References

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